THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

Editorial Board

GARY N. CALKINS, Columbia University
E. G. CONKLIN, Princeton University
E. N. HARVEY, Princeton University
SELIG HECHT, Columbia University
LEIGH HOADLEY, Harvard University
L. IRVING, Swarthmore College
M. H. JACOBS, University of Pennsylvania
H. S. JENNINGS, Johns Hopkins University
FRANK R. LILLIE, University of Chicago
CARL R. MOORE, University of Chicago
GEORGE T. MOORE, Missouri Botanical Garden
T. H. MORGAN, California Institute of Technology
G. H. PARKER, Harvard University
A. C. REDFIELD, Harvard University
F. SCHRADER, Columbia University

H. B. STEINBACH, Washington University
Managing Editor

VOLUME 83

AUGUST TO DECEMBER, 1942

Printed and Issued by
LANCASTER PRESS, Inc.
PRINCE & LEMON STS.
LANCASTER, PA.

THE BIOLOGICAL BULLETIN is issued six times a year. Single numbers, $1.75. Subscription per volume (3 numbers), $4.50.

Subscriptions and other matter should be addressed to the Biological Bulletin, Prince and Lemon Streets, Lancaster, Pa. Agent for Great Britain: Wheldon & Wesley, Limited, 2, 3 and 4 Arthur Street, New Oxford Street, London, W.C. 2.

Communications relative to manuscripts should be sent to the Managing Editor, Marine Biological Laboratory, Woods Hole, Mass., between July 1 and October 1 and to the Department of Zoology, Columbia University, New York City, during the remainder of the year.

Entered as second-class matter May 17, 1930, at the post office at Lancaster, Pa., under the Act of August 24, 1912.

LANCASTER PRESS, INC., LANCASTER, PA.

Science Lib
Mahr

CONTENTS

No. 1. August, 1942

PAGE

Annual Report of the Marine Biological Laboratory..... 1

Kille, Frank R.
Regeneration of the Reproductive System Following Binary Fission in the Sea-Cucumber, Holothuria parvula (Selenka). 55

Johnson, Martin W., and Weldon M. Lewis
Pelagic Larval Stages of the Sand Crabs, Emerita analoga (Stimpson), Blepharipoda occidentalis Randall, and Lepidopa myops Stimpson..... 67

Mahr, Merle M.
Precipitin Reactions and Species Specificity of Monezia expansa and Monezia benedeni..... 88

Andrews, E. A.
Parafolliculina violacea (Giard) at Woods Hole..... 91

Thivy, Francesca
A New Species of Ectochaete (Huber) Wille from Woods Hole, Massachusetts..... 97

Ifft, John D.
The Effect of Environmental Factors on the Sperm Cycle of Triturus viridescens..... 111

Bailey, Basil, P. Koran and H. C. Bradley
The Autolysis of Muscle of Highly Active and Less Active Fish 129

Van Wagtendonk, W. J., F. A. Fuhrman, E. L. Tatum and J. Field, II
Triturus Toxin: Chemical Nature and Effects on Tissue Respiration and Glycolysis..... 137

No. 2. October, 1942

Prosser, C. Ladd
An Analysis of the Action of Acetylcholine on Hearts, Particularly in Arthropods..... 145

Baylor, E. R.
Cardiac Pharmacology of the Cladoceran, Daphnia..... 165

PAGE

MAST, S. O.
The Hydrogen Ion Concentration of the Content of the Food Vacuoles and the Cytoplasm in Amoeba and Other Phenomena Concerning the Food Vacuoles 173

DEFALCO, RALPH J.
A Serological Study of Some Avian Relationships 205

DOUDOROFF, PETER
The Resistance and Acclimatization of Marine Fishes to Experimental Changes. I. Experiments with Girella Nigricans (Ayres) 219

ALLEE, W. C., A. J. FINKEL, H. R. GARNER, G. EVANS, AND R. M. MERWIN
Some Effects of Homotypic Extracts on the Rate of Cleavage of Arbacia Eggs 245

ANDERSON, BERTIL G., AND JOSEPH C. JENKINS
A Time Study of Events in the Life Span of Daphnia 260

VON BRAND, THEODOR, NORRIS W. RAKESTRAW, AND J. WILLIAM ZABOR
Decomposition and Regeneration of Nitrogenous Organic Matter in Sea Water. V. Factors Influencing the Length of the Cycle; Observations upon the Gaseous and Dissolved Organic Nitrogen 273

FORBES, GRACE SPRINGER, AND HENRY E. CRAMPTON
The Effects of Population Density upon Grown and Size in Lymnaea Palustris 283

ABSTRACTS OF SCIENTIFIC PAPERS PRESENTED AT THE MARINE BIOLOGICAL LABORATORY, SUMMER OF 1942 290

NO. 3. DECEMBER, 1942

HUNGATE, R. E.
The Culture of Eudiplodinium neglectum, with Experiments on the Digestion of Cellulose 303

WILBER, CHARLES G.
Digestion of Fat in the Rhizopod, Pelomyxa carolinensis 320

MULLINS, LORIN J.
The Permeability of Yeast Cells to Radiophosphate 326

ELLIS, C. H., C. H. THIENES, AND C. A. G. WIERSMA
The Influence of Certain Drugs on the Crustacean Nerve-muscle System 334

BISHOP, DAVID W.
Oxygen Consumption of Fox Sperm 353

PAGE

HAYWOOD, CHARLOTTE, AND MARY JEANNE CLAPP
A Note on the Freezing-points of the Urines of Two Freshwater Fishes: the Catfish (Ameiurus nebulosus) and the Sucker (Catostomus commersonii) 363

FANKHAUSER, G., AND R. R. HUMPHREY
Induction of Triploidy and Haploidy in Axolotl Eggs by Cold Treatment 367

MOORE, JOHN A.
Embryonic Temperature Tolerance and Rate of Development in Rana catesbiana 375

TURNER, C. L.
Sexual Dimorphism in the Pectoral Fin of Gambusia and the Induction of the Male Character in the Female by Androgenic Hormones 389

REINHARD, EDWARD G.
Studies on the Life History and Host-parasite Relationship of Peltogaster paguri 401

MILLER, JAMES A.
Some Effects of Covering the Perisarc upon Tubularian Regeneration 416

MARTIN, HUGH E., AND LAURA HARE
The Nutritive Requirements of Tenebrio molitor Larvae 428

BUDINGTON, ROBERT A.
The Ciliary Transport System of Asterias forbesi 438

Science Library

Volume 83 Number 1

69

AUG 1 - 1942

THE BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

Editorial Board

GARY N. CALKINS, Columbia University
E. G. CONKLIN, Princeton University
E. N. HARVEY, Princeton University
SELIG HECHT, Columbia University
LEIGH HOADLEY, Harvard University
L. IRVING, Swarthmore College
M. H. JACOBS, University of Pennsylvania
H. S. JENNINGS, Johns Hopkins University
FRANK R. LILLIE, University of Chicago
CARL R. MOORE, University of Chicago
GEORGE T. MOORE, Missouri Botanical Garden
T. H. MORGAN, California Institute of Technology
G. H. PARKER, Harvard University
A. C. REDFIELD, Harvard University
F. SCHRADER, Columbia University

H. B. STEINBACH, Washington University
Managing Editor

AUGUST, 1942

Printed and Issued by
LANCASTER PRESS, Inc.
PRINCE & LEMON STS.
LANCASTER, PA.

MICROFILM SERVICE

THE Library of the Marine Biological Laboratory is now prepared to supply microfilms of material from periodicals included in its extensive list. Through the generosity of Dr. Athertone Seidell, the essential equipment has been set up and put into operation. The service is available to all scientists, the charges based on those established by the "Friends of the Army Medical Library" in Washington. The Staff of the Marine Biological Laboratory Library is anxious to extend the Microfilm Service, particularly at this time when distance makes the Library somewhat inaccessible to many who normally use it.

The Library contains 2366 periodical titles. Although the present world situation has cut down the influx of foreign journals, many are still being received. In order to facilitate the Microfilm Service and to inform scientists of this comprehensive series the Biological Bulletin will publish, as a supplement in the near future, a complete list of the titles of all periodicals held and received, following the alphabetical arrangement established in the Library.

A FEW copies of the Index of Volumes 61 to 80 of the Biological Bulletin, published in October 1941, are still available. The Index contains an alphabetical list of authors, showing titles of papers and abstracts, and a classified index of subjects.

The price of the Index is $2.50, postpaid. The Indexes to Volumes 1 to 60 and Volumes 61 to 80 are now offered at a combination price of $5.00. Orders should be addressed to the Marine Biological Laboratory, Woods Hole, Massachusetts.

Vol. 83, No. 1 August, 1942

THE BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

THE MARINE BIOLOGICAL LABORATORY

FORTY-FOURTH REPORT, FOR THE YEAR 1941—
FIFTY-FOURTH YEAR

I. TRUSTEES AND EXECUTIVE COMMITTEE (AS OF AUGUST 12, 1941) 1
STANDING COMMITTEES 2
II. ACT OF INCORPORATION 3
III. BY-LAWS OF THE CORPORATION 4
IV. REPORT OF THE TREASURER 5
V. REPORT OF THE LIBRARIAN 10
VI. REPORT OF THE DIRECTOR 12
Statement 12
Addenda:
1. Memorials of Deceased Trustees 15
2. The Staff, 1941 19
3. Investigators and Students, 1941 22
4. Tabular View of Attendance 32
5. Subscribing and Co-operating Institutions, 1941 .. 32
6. Evening Lectures, 1941 33
7. Shorter Scientific Papers, 1941 34
8. General Scientific Meetings, 1941 36
9. Members of the Corporation 40

I. TRUSTEES

EX OFFICIO

FRANK R. LILLIE, *President of the Corporation*, The University of Chicago.
CHARLES PACKARD, *Director*, Columbia University.
LAWRASON RIGGS, JR., *Treasurer*, 120 Broadway, New York City.
PHILIP B. ARMSTRONG, *Clerk of the Corporation*, Syracuse University Medical College.

EMERITUS

H. C. BUMPUS, Brown University.
G. N. CALKINS, Columbia University.
E. G. CONKLIN, Princeton University.
CASWELL GRAVE, Washington University.
R. A. HARPER, Columbia University.

Ross G. Harrison, Yale University.
H. S. Jennings, University of California.
C. E. McClung, University of Pennsylvania.
T. H. Morgan, California Institute of Technology.
W. J. V. Osterhout, Rockefeller Institute.
G. H. Parker, Harvard University.
W. B. Scott, Princeton University.

TO SERVE UNTIL 1945

W. R. Amberson, University of Maryland School of Medicine.
S. C. Brooks, University of California.
W. C. Curtis, University of Missouri.
H. B. Goodrich, Wesleyan University.
I. F. Lewis, University of Virginia.
R. S. Lillie, The University of Chicago.
A. C. Redfield, Harvard University.
C. C. Speidel, University of Virginia.

TO SERVE UNTIL 1944

R. Chambers, Washington Square College, New York University.
W. E. Garrey, Vanderbilt University Medical School.
Columbus Iselin, Woods Hole Oceanographic Institution.
S. O. Mast, Johns Hopkins University.
A. P. Mathews, University of Cincinnati.
C. W. Metz, University of Pennsylvania.
H. H. Plough, Amherst College.
W. R. Taylor, University of Michigan.

TO SERVE UNTIL 1943

W. C. Allee, The University of Chicago.
G. H. A. Clowes, Lilly Research Laboratory.
B. M. Duggar, University of Wisconsin.
L. V. Heilbrunn, University of Pennsylvania.
Laurence Irving, Swarthmore College.
J. H. Northrop, Rockefeller Institute.
A. H. Sturtevant, California Institute of Technology.
Lorande L. Woodruff, Yale University.

TO SERVE UNTIL 1942

Dugald E. S. Brown, New York University.
E. R. Clark, University of Pennsylvania.
Otto C. Glaser, Amherst College.
E. N. Harvey, Princeton University.
M. H. Jacobs, University of Pennsylvania.
F. P. Knowlton, Syracuse University.
Franz Schrader, Columbia University.
B. H. Willier, Johns Hopkins University.

EXECUTIVE COMMITTEE OF THE BOARD OF TRUSTEES

Frank R. Lillie, *Ex. Off. Chairman.*
Charles Packard, *Ex. Off.*

LAWRASON RIGGS, JR., *Ex. Off.*
P. B. ARMSTRONG, to serve until 1942.
W. C. ALLEE, to serve until 1942.
D. E. S. BROWN, to serve until 1943.
B. H. WILLIER, to serve until 1943.

THE LIBRARY COMMITTEE

E. G. CONKLIN, *Chairman.*
WILLIAM R. AMBERSON.
C. O. ISELIN, II.
C. C. SPEIDEL.
A. H. STURTEVANT.
WILLIAM R. TAYLOR.

THE APPARATUS COMMITTEE

D. E. S. BROWN, *Chairman.*
W. C. CURTIS.
A. K. PARPART.
F. J. M. SICHEL.

THE SUPPLY DEPARTMENT COMMITTEE

LAURENCE IRVING, *Chairman.*
T. H. BISSONNETTE.
H. B. GOODRICH.
A. C. REDFIELD.
C. C. SPEIDEL.

THE EVENING LECTURE COMMITTEE

B. H. WILLIER, *Chairman.*
M. H. JACOBS.
CHARLES PACKARD.

THE INSTRUCTION COMMITTEE

W. C. ALLEE, *Chairman.*
P. B. ARMSTRONG.
J. B. BUCK.
W. C. CURTIS.
H. B. GOODRICH.
M. H. JACOBS.
CHARLES PACKARD.

II. ACT OF INCORPORATION

No. 3170

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the

purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand Eight Hundred and Eighty-Eight.

[SEAL]

HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 11.30 A.M., daylight saving time, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk to serve one year, and eight Trustees to serve four years. There shall be thirty-two Trustees thus chosen divided into four classes, each to serve four years, and in addition there shall be two groups of Trustees as follows: (*a*) Trustees *ex officio,* who shall be the President of the Corporation, the Director of the Laboratory, the Associate Director, the Treasurer and the Clerk; (*b*) Trustees Emeritus, who shall be elected from the Trustees by the Corporation. Any regular Trustee who has attained the age of seventy years shall continue to serve as Trustee until the next annual meeting of the Corporation, whereupon his office as regular Trustee shall become vacant and be filled by election by the Corporation and he shall become eligible for election as Trustee Emeritus for life. The Trustees *ex officio* and Emeritus shall have all rights of the Trustees except that Trustees Emeritus shall not have the right to vote.

The Trustees and officers shall hold their respective offices until their successors are chosen and have qualified in their stead.

II. Special meetings of the members may be called by the Trustees to be held in Boston or in Woods Hole at such time and place as may be designated.

III. Inasmuch as the time and place of the Annual Meeting of Members are fixed by these By-laws, no notice of the Annual Meeting need be given.

Notice of any special meeting of members, however, shall be given by the Clerk by mailing notice of the time and place and purpose of said meeting, at least fifteen (15) days before such meeting, to each member at his or her address as shown on the records of the Corporation.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President of the Corporation who shall also be Chairman of the Board of Trustees; they shall appoint a Director of the Laboratory; and they may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The accounts of the Treasurer shall be audited annually by a certified public accountant.

VIII. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees.

IX. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. THE REPORT OF THE TREASURER

To the Trustees of the Marine Biological Laboratory:

Gentlemen:

Herewith is my report as Treasurer of the Marine Biological Laboratory for the year 1941.

The accounts have been audited by Messrs. Seamans, Stetson and Tuttle, certified public accountants. A copy of their report is on file at the Laboratory and is open to inspection by members of the Corporation.

At the end of the year 1941, the book value of the Endowment Fund in the hands of the Central Hanover Bank and Trust Company, as

Trustee, was

General Fund,	Securities (Market $749,361.44)	$803,267.92
	Real Estate	74,835.98
	Cash	2,719.28
		$880,823.18
Library Fund,	Securities (Market $152,425.11)	$169,425.16
	Real Estate	20,000.00
	Cash	258.05
		$189,683.21

The income collected from these funds during the year was:

General Endowment	$32,306.81
Library	6,572.92
	$38,879.73

The income in arrears on these funds at the end of the year was:

General Fund	$ 6,300.00
Library	$ 5,231.00
	$11,531.00
Last year	$16,578.69

This reduction does not represent the payment of back interest, $6,750, being accounted for by interest written off on a foreclosure of a mortgage.

General Biological Supply House, Inc.: The dividends from General Biological Supply House, Inc. totalled $17,780, a decrease of $762 from 1940.

Bar Neck Property: The rental from the Bar Neck property which is based on the net profit of the garage was $4,719.28.

Retirement Fund: A total of $3,460 was paid in pensions. This fund at the end of the year consisted of:

Participations in mortgages	$ 7,464.99
Interest in Real Estate	2,301.88
Cash	5,046.91
	$14,813.78

Plant Assets: The land (exclusive of Gansett and Devil's Lane Tracts), the buildings, equipment and library represent an investment

of	$1,926,531.07	
less reserve for depreciation	582,468.84	
or a net of		$1,344,062.23

Income and Expenses: The expenses including Reserve for Depreciation of $25,603.89 exceeded income by $9,482.01. There was expended from current funds for plant account a net of $16,248.50 and $1,748.86 net, was added to the Reserve Fund.

At the end of the year the Laboratory had no indebtedness on notes or mortgages. It owed on accounts payable $6,423.47, against which it had accounts receivable of $14,585.69 and cash in its general bank accounts of $10,619.85.

$45,623.38 was received during the year from the Rockefeller Foundation for the building of the library annex which completed its grant of $110,400, and there was expended during the year $67,714.81, which with the expenditures of 1940 brought the total cost of the building at the end of the year to $107,565.93, leaving a balance in the Building Fund account of $2,834.07.

$25,000 was received from The Carnegie Corporation of New York for the purchase of books and back sets and the improvement of the library.

Following is the balance sheet, the condensed statement of income and outgo and the surplus account all as set out by the auditors.

EXHIBIT A

Marine Biological Laboratory Balance Sheet
December 31, 1941

Assets

Endowment Assets and Equities:			
Securities and Cash in Hands of Central Hanover Bank and Trust Company, New York, Trustee —Schedules I-a and I-b		$1,070,506.39	
Securities and Cash—Minor Funds—Schedule II		9,305.68	$1,079,812.07
Plant Assets:			
Land—Schedule IV	$ 111,425.38		
Buildings—Schedule IV	1,319,364.52		
Equipment—Schedule IV	185,610.60		
Library—Schedule IV	310,130.57		
	$1,926,531.07		
Less Reserve for Depreciation	582,468.84	$1,344,062.23	
Cash in Building Fund		2,834.07	
Cash in Reserve Fund		4,273.51	
Cash in Book Fund		25,000.00	$1,376,169.81

Current Assets:			
Cash		$ 10,619.85	
Accounts-Receivable		14,585.69	
Inventories:			
Supply Department	$ 38,658.35		
Biological Bulletin	12,228.56	50,886.91	
Investments:			
Devil's Lane Property	$ 45,375.48		
Gansett Property	998.70		
Stock in General Biological Supply House, Inc.	12,700.00		
Other Investment Stocks	17,770.00		
Securities, Real Estate, and Cash —Retiremend Fund—Schedule V	14,813.78	91,657.96	
Prepaid Insurance		3,761.21	
Items in Suspense		159.09	$ 171,670.71
Liabilities			
Endowment Funds:			
Endowment Funds—Schedule III	$1,068,780.68		
Reserve for Amortization of Bond Premiums	1,725.71	$1,070,506.39	
Minor Funds—Schedule III		9,305.68	$1,079,812.07
Plant Liabilities and Surplus:			
Donations and Gifts—Schedule III		$1,175,290.11	
Other Investments in Plant from Gifts and Current Funds		200,879.70	$1,376,169.81
Current Liabilities and Surplus:			
Accounts—Payable		$ 6,423.47	
Current Surplus—Exhibit C		165,247.24	$ 171,670.71

EXHIBIT B

Marine Biological Laboratory Income and Expense, Year Ended December 31, 1941

	Total		Net	
	Expense	Income	Expense	Income
Income:				
General Endowment Fund		$ 32,306.81		$ 32,306.81
Library Fund		6,572.92		6,572.92
Donations		100.00		100.00
Instruction	$ 9,593.55	9,770.00		176.45
Research	6,130.85	14,737.50		8,606.65
Evening Lectures	108.48		$ 108.48	
Biological Bulletin and Membership Dues	8,232.34	9,625.18		1,392.84

Supply Department—Schedule VI ..	33,600.35	30,527.78	3,072.57	
Mess—Schedule VII	23,502.23	21,460.17	2,042.06	
Dormitories—Schedule VIII	23,994.53	12,417.10	11,577.43	
(Interest and Depreciation charged to above 3 Departments—See Schedules VI, VII, and VIII)	24,117.65			24,117.65
Dividends, General Biological Supply House, Inc.		17,780.00		17,780.00
Dividends, Crane Company		500.00		500.00
Rents,				
Bar Neck Property		4,719.28		4,719.28
Janitor House	115.61	360.00		244.39
Danchakoff Cottages	367.13	700.00		332.87
Sale of Library Duplicates		87.43		87.43
Apparatus Rental		925.38		925.38
Sundry Income		157.15		157.15
Maintenance of Plant:				
Buildings and Grounds	25,221.68		25,221.68	
Chemical and Special Apparatus Expense	16,772.95		16,772.95	
Library Expense	7,776.09		7,776.09	
Workmen's Compensation Insurance	528.09		528.09	
Truck Expense	497.11		497.11	
Bay Shore Property	126.10		126.10	
Great Cedar Swamp	158.88		158.88	
General Expenses:				
Administration Expense	12,295.53		12,295.53	
Endowment Fund Trustee and Safe-keeping	1,014.45		1,014.45	
Bad Debts	706.52		706.52	
Reserve for Depreciation	25,603.89		25,603.89	
	$172,228.71	$162.746.70	$107,501.83	$ 98,019.82
Excess of Expense over Income carried to Current Surplus—Exhibit C		9,482.01		9,482.01
		$172,228.71		$107,501.83

EXHIBIT C

Marine Biological Laboratory, Current Surplus Account
Year Ended December 31, 1941

Balance, January 1, 1941		$172,107.36
Add:		
Reserve for Depreciation Charged to Plant Funds	$25,603.89	
Bad Debt Recovered	6.00	
Gain on Gansett Lots Sold	450.88	26,060.77
		$198,168.13

Deduct:			
Excess of Expense over Income for Year as shown in Exhibit B		$ 9,482.01	
Payments from Current Funds during Year for Plant Assets as shown in Schedule IV,			
Buildings	$ 4,167.02		
Equipment	3,298.84		
Library	9,188.24		
	$16,654.10		
Less Received for Plant Assets Disposed of	405.60	16,248.50	
Transfer to Plant Reserve Fund		3,977.18	
Pensions Paid	$ 3,460.00		
Less Retirement Fund Income	246.80	3,213.20	$ 32,920.89
Balance, December 31, 1941—Exhibit A			$165,247.24

Respectfully submitted,

LAWRASON RIGGS,
Treasurer.

V. REPORT OF THE LIBRARIAN

The appropriation to the Library for 1941 of $18,850.00 was expended as follows: books, $765.45; serials, $2739.53; binding, $1177.92; express, $90.60; supplies, $509.51; salaries, $7200.00; back sets, $3717.83; and sundries, $73.11, leaving a balance of $2663.48 (counting the duplicate sales which amounted to $87.43). From the sum held in reserve from the 1940 appropriation (see report of 1940) $2228.32 was used leaving a balance of $1748.86.

In December 1941 the gift of $25,000 from the Carnegie Corporation was appropriated to the Marine Biological Laboratory for Library development. This sum will not be subject to reversion to source and will be available whenever the foreign market for purchases is again open. In the meantime purchases are restricted to this country and to England where the rare sets we need are practically unavailable.

The Woods Hole Oceanographic Institution appropriated $800.00 to the Library for 1941 and a balance of $133.75 remained from the 1940 budget. An expended sum of $779.10 has been reported to the Director.

The Library receives currently 1297 serial publications: 409 (12 new) subscriptions to the Marine Biological Laboratory, 40 (6 new) to the Woods Hole Oceanographic Institution; 630 exchanges, 558 (13 new) with "The Biological Bulletin" and 72 (2 new) with the Woods Hole Oceanographic Institution publications; 210 gifts to the former institution and 8 to the latter. The Marine Biological Laboratory acquired 222 books: 86 by purchase of the Marine Biological Laboratory,

23 by purchase of the Woods Hole Oceanographic Institution, 7 presented by Dr. F. B. Hanson, 12 gifts from the authors, 58 gifts of publishers, 5 acquired by duplicate exchange, and 31 by miscellaneous donors. There were 27 back sets of serial publications completed: by purchase of the Marine Biological Laboratory, 13, by the Woods Hole Oceanographic Institution, 3, by exchange with "The Biological Bulletin," 5, by exchange of duplicates, 2, by gift, 4. Partially completed sets were 94: by purchase of the Marine Biological Laboratory, 12, of the Woods Hole Oceanographic Institution, 2, by exchange with "The Biological Bulletin," 5, with the Woods Hole Oceanographic Institution, 1, by exchange of duplicates, 34, and by gift, 40. Reprint additions number 3321: current of 1940, 1250; current of 1941, 552; and of previous dates, 1519. The present holdings of the Library are 49,179 bound volumes and 119,626 reprints.

The gifts of reprint collections have been very generous in the past two years. In 1940 Mrs. Metcalf presented 100 reprints and 2 books to be added to the Metcalf collection already here. Only 60 of these were duplicates. Dr. R. P. Bigelow gave us 1400 reprints, 450 of which were new for us. The Director of the Museum of Comparative Zoology presented a reprint of the "Origin and progress of the Anderson School of Natural History, Penikese Island, 1874." In 1941, Dr. Frank R. Lillie presented 29 reprints (4 not duplicated); Dr. Libbie Hyman, 270 (95 not duplicated); Dr. S. Wing Handford, 181 (72 not duplicated); Dr. E. W. Gudger, 23 (8 not duplicated); Dr. G. S. Dodds, 577 (192 not duplicated); Dr. Robert Chambers, 685 (221 not duplicated); Dr. G. N. Calkins, 750 (210 not duplicated); Dr. R. P. Bigelow, 3017 (873 not duplicated).

It seems not out of place at this date of sending in the 1941 report (March, 1942) to give important information that properly belongs in the 1942 report of the Library. A gift of a complete microfilming outfit has been presented and installed, and instruction given to a member of the library staff. Dr. Atherton Seidell of "Medicofilm Service" and the "Current List of Medical Literature" who conducts this service under the auspices of the "Friends of the Army Medical Library" not only presented the apparatus, one of his own construction, but brought the equipment in person and spent four days at the Library to set it up and to train a member of the staff so that she is able to carry on the work at once. In the near future an announcement of the service and a list of the journals available in this Library will be issued but in the meantime the acknowledgment of Dr. Seidell's generous gift will help to make known to our investigators this new service of the Library.

VI. THE REPORT OF THE DIRECTOR

To the Trustees of the Marine Biological Laboratory:

Gentlemen:

I present herewith a report of the fifty-fourth session of the Marine Biological Laboratory for the year 1941, with comments on matters which affect the session of 1942.

The war has brought about changes which have already profoundly affected the Laboratory and which will undoubtedly affect it still more in the future. We are faced with a marked decrease in attendance, a very considerable loss in income, and with the difficulty of obtaining supplies for research and for the upkeep of our buildings. But the fine spirit shown by our workers, both investigators and members of the permanent staff, is ample evidence that the Laboratory can adapt itself to its altered environment, and will continue to serve the cause of biological research and instruction.

1. *Attendance.* We shall miss many of our regular attendants who must remain at home to take their part in the accelerated teaching program, to engage in research problems suggested by the Defense Council, or to carry on other work directly connected with the war effort. A considerable number of students who had anticipated entering our courses will continue their studies in the summer sessions of their colleges. Others will enter military service. Many are prevented from coming to Woods Hole by the rubber and gas shortage. As long as the war continues we must expect further declines in attendance.

2. *Income.* The prospect of a sharply reduced income in 1942 is a matter of grave concern. Although our income from all sources has been decreasing steadily for some time and was expected to drop still further, we did not anticipate the very considerable loss with which we are now faced. This loss is due in part to the decreased attendance resulting in smaller returns from investigators' and students' fees, and from the dormitories and the Mess. Interest on the Endowment Funds has grown smaller, and the return from other investments is less. It is estimated that the total income for 1942 may be $20,000 less than that of 1941.

3. *The Budget.* This situation forced the Executive Committee to revise the budget provisionally adopted in September 1941. Appropriations for the various departments have been greatly curtailed. The Library is provided with only enough to pay for the subscriptions to all journals currently received, and for binding. Very little is available for the purchase of back sets. The sum usually reserved for new apparatus

has also been cut, on the assumption that our present supplies are adequate for the time being. It should be noted that it is extremely difficult to buy new apparatus under any conditions. Reductions have also been made in the summer Collecting Crew, in the Chemical Room staff, and in the janitorial service. These changes should not materially affect actual research, but may cause occasional minor delays.

4. *Courses of Instruction.* It was evident to the Committee that the number of students who would apply for entrance to the courses in 1942 would probably be small, and that instruction could be maintained only at a considerable loss unless the expense, that is, the instructors' salaries, could be reduced. The members of the teaching staff were therefore asked if they would be willing to accept a 50 per cent cut, provided a total of only 50 students was admitted. The response to this unwelcome proposition was most heartening, the opinion being expressed that the courses should not be allowed to lapse even for a single summer. The Corporation is deeply indebted to the teaching staff for their willingness to carry on this fundamental part of our program at the risk of financial sacrifice.

5. *Use of Buildings by the Navy.* During the winter we received assurances that none of our buildings would be required by the rapidly expanding Coast Guard stationed at Woods Hole. But late in the spring the Navy Department asked for the exclusive use of the Mess, Homestead, Lecture Hall, and Botany Building. Subsequently the Apartment House was added to the list. These buildings will be occupied for the duration of the war. There was, of course, no hesitation on the part of the Committee in granting this request. To give up the buildings is a contribution to the war effort which we gladly make. The Lecture Hall and Botany could readily be relinquished for the work usually carried on in them can be transferred to other buildings which will not be fully occupied. More disturbing is the loss of the Mess and Apartment House. We are fortunate, however, in securing the Avery Hotel as a Mess. Although its kitchen facilities are not as complete nor as convenient as our own, they can be made to serve satisfactorily under the competent management of Miss Downing, our newly appointed matron.

6. *Gifts.* The Laboratory is deeply indebted to the Carnegie Corporation of New York for its gift of $25,000 to be used for the purchase of back sets of journals, of volumes to fill gaps in our present files of serials, and of classics in biology and allied sciences. By virtue of this timely contribution we are now able to satisfy the terms under which the Rockefeller Foundation granted us funds for the erection of the new wing of the Library. While it is difficult, under present conditions, to make rapid progress in completing our files, we are now assured that

whenever the needed volumes appear in the market, we can secure them. The value of the Library will be greatly enhanced by this gift.

Its usefulness is still further increased by the generosity of Mr. Atherton Seidell of the National Bureau of Health. Mr. Seidell, who has long been active in extending the use of microfilms, presented to the Laboratory a complete photographic and developing outfit for making these films, and helped to install it in one of the dark rooms in the basement of the new wing. He also provided us with a reading instrument. The Library is thus in a position to render valuable service to investigators who cannot secure the references they want without making a journey to some large institution. We are grateful to Mr. Seidell for his extremely useful gift.

7. *The Apparatus Department.* Last fall Dr. S. E. Pond resigned after serving nearly ten years as Technical Manager in charge of Chemicals and Scientific Apparatus, during which time he had greatly expanded the usefulness of his department. The Apparatus Committee, faced with the difficult task of finding a man to replace him, decided to divide the work into two sections, each under the direction of a part-time manager. Dr. E. P. Little, who has had supervision over the X-ray and photographic rooms, was put in charge of the apparatus, and Mr. K. S. Ballard, long a member of the Chemical Room staff, was put in charge of the chemicals and glassware. Dr. Little, with Dr. Sichel as consultant, takes up his work at a time when new apparatus is hard to come by, and when the present stocks must be made to serve—a difficult task but one in which we are confident he will succeed.

8. *Other Items.* Among various items of interest that may be mentioned are:

(*a*) The increase in the price of board at the Mess to $8.00 per week from $7.00, a rate which until now has stood unchanged for more than thirty years. The change was made necessary because last year the Mess was run at a loss.

(*b*) The construction of a new salt water intake pier, replacing the old one which was in imminent danger of collapse. A constant supply of sea water is the most essential part of our equipment for without it practically all research here would come to an end in a few hours. Fortunately, the work was begun in the fall when the necessary materials could still be secured.

(*c*) The sale of ten lots in the Gansett tract. At present only four parcels in this property remain unsold. The Devil's Lane tract, on the Shore Road to Falmouth, will provide space for many of our investigators and must be opened up as soon as there is a sufficient demand.

9. *Election of Officers and Trustees.* At the meeting of the Corporation held August 1, 1941 the following Trustee was elected Trustee Emeritus:

W. J. V. Osterhout, Rockefeller Institute

The new Trustees elected at that meeting were:

G. H. A. Clowes, Class of 1943
Columbus Iselin, Class of 1944

10. There are appended as parts of this report:

1. Memorials of deceased Trustees.
2. The Staff, 1941.
3. Investigators and Students, 1941.
4. A Tabular View of Attendance, 1937–1941.
5. Subscribing and Co-operating Institutions, 1941.
6. Evening Lectures, 1941.
7. Shorter Scientific Papers, 1941.
8. The General Scientific Meeting, 1941.
9. Members of the Corporation, 1941.

Respectfully submitted,

CHARLES PACKARD,
Director.

1. MEMORIALS OF DECEASED TRUSTEES

MEMORIAL TO DR. E. B. MEIGS

BY DR. R. S. LILLIE

Edward Browning Meigs was born in Philadelphia September 10, 1879, the son of Arthur Vincent Meigs and Mary Roberts Browning. He belonged to an old and distinguished American family of South English ancestry, and was a direct descendant of Vincent Meigs who came to America and settled in New Haven, Connecticut, in 1644. His father, grandfather, and great-grandfather were physicians; his great-great-grandfather, Josiah Meigs, was Professor of Mathematics and Natural Philosophy in Yale University in the 1790's. Scientific interests were strong in his ancestry. His father, a pediatrician, was interested in the chemical composition of milk and published papers on this subject; he also introduced a method of modifying cows' milk to make it suitable for infants. In Edward Meigs' memoir of his father he "finds it difficult to say whether more of my father's energy was devoted to the practice of medicine or to research."

Edward Meigs was the fourth physician in his family in the direct line, but his own interests were primarily scientific and he did not engage in practice. He graduated from Princeton University in 1900 and took his M.D. at the University of Pennsylvania in 1904. He was Assistant in Physiology in the same University during 1904–1906 and then spent a year abroad in study and research, working chiefly in Jena with the comparative physiologist, Wilhelm Biedermann. He also spent some time in Cambridge University, chiefly in association with Walter Fletcher and Gowland Hopkins, whose work on the physiology and biochemistry of muscular contraction was of special interest to him. He was instructor in Physiology in the Harvard Medical School during 1907–10. In 1910 he joined the Wistar Institute in Philadelphia in order to devote himself entirely to research. From 1915 until his death he was physiologist in the Bureau of Dairy Industry of the U. S. Department of Agriculture.

He first attended the Marine Biological Laboratory in 1904, and was elected a member of the Corporation in 1905. During the four summers of 1912–1915 he served as instructor in the Physiology course. He and his family have been summer residents of Woods Hole for a period of about thirty years, and his interest in the Laboratory has been constant.

In 1910 he married Margaret Wister of Philadelphia who with his two sons and two daughters survives him. He died November 5, 1940, after a prolonged illness.

Edward Meigs' early investigations were in the field of general physiology, especially the physiology of muscular contraction. Later, after he went to Washington, his work had reference chiefly to the physiology of milk production and related topics; problems connected with administration and the organization of research in this field also engaged much of his attention.

His early studies on the comparative histology and biochemistry of smooth and striated muscle, both vertebrate and invertebrate, were varied and extensive. His photographs of striated muscle fibres under high magnification are among the best that we have. His belief that changes of tension in muscle were a result of reversible changes of hydration in the fibrils led him to experiment on the influence of variations of osmotic pressure, chemical conditions and temperature on the water content and correlated state of contraction of different types of muscle. At one time he was greatly interested in physical models of muscular contraction, especially McDougall's model, in which increase of volume of inflation resulted in shortening. His interest in the relation of inorganic salts to contraction (as shown, e.g., in the potassium contraction of striated

muscle) led him to make comparative analytical studies of the salt content of smooth and striated muscle, vertebrate and invertebrate. This work had an indirect but important bearing on his later work in the Department of Agriculture on mineral metabolism in its relation to milk production. He recognized that the selective action of the muscle cell in accumulating its highly special salt content had the same physiological basis as the selective separation of salts by the mammary gland in milk secretion. Problems of permeability also interested him in relation to both the properties of muscle and the processes of secretion; and in work on artificial membranes he showed that impregnation of collodion films with insoluble calcium and magnesium salts formed membranes approaching living plasma membranes in their semipermeability, a property which in the living cell also is dependent on calcium. He found, however, that the behavior of smooth muscle in anisotonic Ringer's solution differed from that of striated muscle and indicated the presence of a much less diffusion-proof surface layer. This difference in physical properties he correlated with other evidence of a fundamental difference in the mechanism of contraction in the two types of muscle.

During Edward Meigs' work of twenty-five years in the Department of Agriculture he and his associates made varied and important contributions to the physiology of milk production. Mineral metabolism and vitamine supply in relation to milk production received special attention, and the results of this work were published in a long succession of special papers and reviews. He was also responsible for the general planning and direction of the work at the experimental farm at Beltsville, Maryland. Many problems of a highly practical kind also came up for consideration; for example, the incidence of mastitis in the experimental herd led to an investigation of the pathology of this condition and effective methods for its control were developed, including modification of certain types of milking machines which were found to be largely responsible. The work of these years is too varied to summarize briefly; some of its practical results are seen in the progressive improvement during recent years in the general methods employed in the dairy industry.

Much of Edward Meigs' success in this work came from the thoroughness, objectivity and freedom from bias that were characteristic of his scientific activity and outlook. He was highly tenacious in his convictions once they were formed, but his conclusions were always based on a clear-sighted and critical consideration of evidence, in the collection of which he spared no pains. Although weakened in his later years by illness, he maintained his scientific interest and activity to the last. His personal interests other than scientific were remarkably wide; he was

fond of nature and outdoor life, a great sailor, a man of imagination and culture, widely read, modest, loyal, high-minded and devoted to his friends. His characteristic generosity was well shown in his gift to the Marine Biological Laboratory in 1936 of the bathing beach property, including the bath house, on the Buzzards Bay front adjoining his family summer cottage. The use of this beach by members of the Laboratory, as well as of the tennis courts which occupy part of this property, is thus permanently assured.

Memorial to Dr. D. H. Tennent

By Dr. W. C. Curtis

In the premature death of David Hilt Tennent, the Corporation of the Marine Biological Laboratory has lost the crowning years in the life of a member distinguished for his accomplishment in research and even more for his quality as a man. Tennent was an investigator who proceeded without haste, yet unceasingly; for him quality, not quantity, of publication was the prime consideration, yet the volume of his published work is impressive. He was honored for his work by the Presidency of the American Society of Zoologists and by similar offices, and most notably by election to the National Academy of Science. He made outstanding contributions in his studies upon hybridization, fertilization and egg organization in Echinoderms, and in his later research upon photosensitization in which he took especial satisfaction since he regarded it as the most important work of his life. These contributions, which are familiar to workers in these fields, are not so well known to many investigators in other lines, because Tennent was the most modest and retiring of men. He had none of the flare for self-advertisement that carries some men so far on a modicum of worth. His every publication was marked not only by the critical nature of his observations and experiments but also by the meticulous care with which each phrase weighed to make sure it meant exactly what he had in mind, no more and no less. What he wrote or said publicly was always as exact as he could make it. Knowing the quality of the man and of his mind, I think one may feel that what he did is likely to stand until it becomes obsolete with the advance of knowledge, as so often happens although the historical importance of the work remains.

In his work as a teacher of undergraduates and as a director of graduate students, Tennent was no less effective. The same thoroughness and determination to do his best characterized his teaching as it did his research. Tennent and I were graduate students together at the Johns Hopkins and fellow members of Drew's Invertebrate staff at the

Marine Biological Laboratory. I well recall his first lecture to the Invertebrate class. He was so scared the chalk rattled against the board, but he did better than he thought and after that first summer the rest of us felt we must keep up with him. At the Hopkins he was not satisfied with his first year's seminar lectures. To be safe the next year, as he confided to me later, he went to the laboratory the evening before each lecture, turned on the lights in the empty seminar room and put himself through a dress rehearsal of his lecture to be given the next morning. It was this kind of determination and performance that characterized all his work. It had to be done as well as he could do it. I was told that E. A. Andrews went so far as to say at the time that Tennent was the best assistant he had ever had. Only those of us who were assistants to Andrews can fully appreciate what that meant as to quality of performance. Thus, Tennent had the instinct of workmanship at the beginning of his career.

Tennent always commanded the loyalty and admiration of graduate students to a marked degree. His great disappointment was that he did not train more students who were able to find places commensurate with their ability. Those in his confidence knew that he often longed for a position where he might have had more "disciples." He trained many women of ability, but for the most part, in this *man's* world, they could not find positions worthy of their competence. His summers at the Tortugas Laboratory gave him opportunities to extend the kind of contacts he might have had in larger measure throughout the year in some institutions. I have often heard of what a stimulus he was to the younger investigators at Tortugas summer after summer.

On the personal side, Tennent was always quiet and reserved, though in his later years as well as in his youth a delightful companion to those who knew him well. He may have seemed austere to those who knew him casually. Yet he had a keen sense of humor for all his quietness. I never knew a man whose sense of obligation to do what he thought just and right seemed to me stronger nor a man whom I would trust further. Thinking of him personally, one felt that here was a man to whom the abused and meaningless phrase "a gentleman and a scholar" might be applied with meaning.

To Mrs. Tennent and to his son, who as a scientist follows in his father's footsteps, we extend our deepest sympathy.

2. THE STAFF, 1941

CHARLES PACKARD, Director, Assistant Professor of Zoology, Institute of Cancer Research, Columbia University.

ZOOLOGY

I. Investigation

Gary N. Calkins, Professor of Protozoology, Emeritus, Columbia University.
E. G. Conklin, Professor of Zoology, Emeritus, Princeton University.
Caswell Grave, Professor of Zoology, Emeritus, Washington University.
Frank R. Lillie, Professor of Embryology, Emeritus, The University of Chicago.
C. E. McClung, Professor of Zoology, Emeritus, University of Pennsylvania.
S. O. Mast, Professor of Zoology, Johns Hopkins University.
T. H. Morgan, Director of the Biological Laboratory, California Institute of Technology.
G. H. Parker, Professor of Zoology, Emeritus, Harvard University.
Lorande L. Woodruff, Professor of Protozoology, Yale University.

II. Instruction

T. H. Bissonnette, Professor of Biology, Trinity College.
P. S. Crowell, Jr., Instructor in Zoology, Miami University.
A. M. Lucas, Associate Professor of Zoology, Iowa State College.
W. E. Martin, Assistant Professor of Zoology, DePauw University.
N. T. Mattox, Instructor in Zoology, Miami University.
J. S. Rankin, Jr., Instructor in Biology, Amherst College.
A. J. Waterman, Assistant Professor of Biology, Williams College.

Junior Instructors

W. J. Bowen, Assistant Professor of Biology, University of North Carolina.
E. R. Jones, Jr., Professor of Biology, College of William and Mary.

EMBRYOLOGY

I. Investigation

(*See Zoology*)

II. Instruction

Hubert B. Goodrich, Professor of Biology, Wesleyan University.
W. W. Ballard, Assistant Professor of Biology and Anatomy, Dartmouth College.
Donald P. Costello, Assistant Professor of Zoology, University of North Carolina.
Viktor Hamburger, Assistant Professor of Zoology, Washington University.
Oscar Schotté, Associate Professor of Biology, Amherst College.

PHYSIOLOGY

I. Investigation

William R. Amberson, Professor of Physiology, University of Maryland, School of Medicine.

HAROLD C. BRADLEY, Professor of Physiological Chemistry, University of Wisconsin.
WALTER E. GARREY, Professor of Physiology, Vanderbilt University Medical School.
M. H. JACOBS, Professor of Physiology, University of Pennsylvania.
RALPH S. LILLIE, Professor of General Physiology, The University of Chicago.
ALBERT P. MATHEWS, Professor of Biochemistry, University of Cincinnati.

II. INSTRUCTION

Teaching Staff

ARTHUR K. PARPART, Associate Professor of Biology, Princeton University, in charge of course.
ROBERT BALLENTINE, National Research Council Fellow, Rockefeller Institute, New York.
KENNETH C. FISHER, Assistant Professor of Experimental Biology, University of Toronto.
RUDOLF T. KEMPTON, Professor of Zoology, Vassar College.
C. LADD PROSSER, Assistant Professor of Zoology, University of Illinois.
F. J. M. SICHEL, Assistant Professor of Physiology, University of Vermont, College of Medicine.

BOTANY

I. INVESTIGATION

S. C. BROOKS, Professor of Zoology, University of California.
B. M. DUGGAR, Professor of Physiological and Economic Botany, University of Wisconsin.
D. R. GODDARD, Assistant Professor of Botany, University of Rochester.
E. W. SINNOTT, Professor of Botany, Barnard College.

II. INSTRUCTION

WM. RANDOLPH TAYLOR, Professor of Botany, University of Michigan.
B. F. D. RUNK, Instructor in Botany, University of Virginia.
WILLIAM J. GILBERT, Department of Botany, University of Michigan.

GENERAL OFFICE

F. M. MACNAUGHT, Business Manager.
POLLY L. CROWELL, Assistant.
GLADE ALLEN, Secretary.

RESEARCH SERVICE AND GENERAL MAINTENANCE

SAMUEL E. POND, Technical Mgr.
G. FAILLA, X-ray Physicist.
ELBERT P. LITTLE, X-ray Technician.
J. D. GRAHAM, Glassblower.
T. E. LARKIN, Superintendent.
LESTER F. BOSS, Technician.
W. C. HEMENWAY, Carpenter.
R. S. LILJESTRAND.

LIBRARY

PRISCILLA B. MONTGOMERY (Mrs. Thomas H. Montgomery, Jr.) Librarian.
DEBORAH LAWRENCE, Secretary.
MARY A. ROHAN, S. MABELL THOMBS, Assistants.

SUPPLY DEPARTMENT

JAMES McINNIS, Manager.
MILTON B. GRAY, Collector.
A. M. HILTON, Collector.
A. W. LEATHERS, Shipping Dept.
GEOFFREY LEHY, Collector.
WALTER KAHLER, Collector.
F. N. WHITMAN, Collector.
RUTH S. CROWELL, Secretary.
GRACE HARMAN, Secretary.

INVESTIGATORS AND STUDENTS

Independent Investigators, 1941

ADDISON, WILLIAM H. F., Professor of Normal Histology and Embryology, University of Pennsylvania, School of Medicine.
ALLEE, W. C., Professor of Zoology, The University of Chicago.
AMBERSON, WILLIAM R., Professor of Physiology, University of Maryland, School of Medicine.
BAKER, HORACE B., Professor of Zoology, University of Pennsylvania.
BALLARD, W. W., Assistant Professor of Biology and Anatomy, Dartmouth College.
BALLENTINE, ROBERT, Procter Fellow, Princeton University.
BARRON, E. S. GUZMAN, Assistant Professor of Biochemistry, The University of Chicago.
BARTLETT, JAMES H., JR., Associate Professor of Theoretical Physics, University of Illinois.
BERGER, CHARLES A., Professor of Cytology, Director of Biological Laboratory, Fordham University.
BISSONNETTE, T. H., Professor of Biology and Head of Biology Department, Trinity College.
BLISS, ALFRED F., Lecturer in Biophysics, Columbia University.
BOCHE, R. D., Instructor in Zoology, University of Pennsylvania.
BOWEN, W. J., Assistant Professor, University of North Carolina.
BOYD, MILFORD J., Assistant Professor of Biochemistry, University of Cincinnati.
BRADLEY, HAROLD C., Professor of Physiological Chemistry, University of Wisconsin.
BRILL, EDMUND R., Graduate Student in Biology, Harvard University.
BRONFENBRENNER, J. J., Professor of Bacteriology and Immunology, Washington University.
BROOKS, MATILDA M., Research Associate, University of California.
BROOKS, SUMNER C., Professor of Zoology, University of California.
BROWN, DUGALD E. S., Professor of Physiology, New York University.
BUDINGTON, R. A., Professor of Zoology, Emeritus, Oberlin College.
BULLOCK, THEODORE H., Sterling Fellow in Zoology, Yale University.
CABLE, R. M., Associate Professor of Parasitology, Purdue University.
CALKINS, GARY N., Professor Emeritus of Protozoology, Columbia University.
CAROTHERS, E. ELEANOR, Research Associate in Zoology, University of Iowa.
CHAMBERS, EDWARD, Research Worker, Washington Square College, New York University.
CHAMBERS, ROBERT, Research Professor of Biology, Washington Square College, New York University.

CHASE, AURIN M., Instructor, Princeton University.
CLAFF, C. LLOYD, Research Associate in Biology, Brown University.
CLARK, ELEANOR LINTON, Voluntary Research Worker, University of Pennsylvania.
CLARK, ELIOT R., Professor and Director of Department of Anatomy, University of Pennsylvania, School of Medicine.
CLARK, LEONARD B., Assistant Professor of Biology, Union College.
CLAUDE, ALBERT, Research, Rockefeller Institute.
CLOWES, G. H. A., Director of Research, Eli Lilly and Company.
COLE, KENNETH S., Associate Professor of Physiology, Columbia University.
COLWIN, ARTHUR L., Instructor, Queens College.
COLWIN, LAURA HUNTER, Instructor, Vassar College.
CONKLIN, EDWIN G., Professor of Biology, Emeritus, Princeton University.
COOPER, KENNETH W., Instructor, Princeton University.
COOPER, RUTH, Research Worker, Princeton University.
COPELAND, MANTON, Professor of Biology, Bowdoin College.
CORNMAN, IVOR, Graduate Student, University of Michigan.
COSTELLO, DONALD P., Assistant Professor of Zoology, University of North Carolina.
CROWELL, SEARS, Assistant Professor of Zoology, Miami University.
CURTIS, W. C., Professor of Zoology, University of Missouri.
DAM, HENRIK, Associate Professor of Biochemistry, University of Copenhagen.
DAVSON, HUGH, Associate Professor of Physiology, Dalhousie University.
DEWEY, VIRGINIA C., Manufacturers' Research Fellow in Protozoology, Brown University.
DONNELLON, JAMES A., Assistant Professor of Biology, Villanova College.
DUBOIS, EUGENE F., Professor of Medicine, Cornell University Medical College.
DUGGAR, B. M., Professor of Plant Physiology and Applied Botany, University of Wisconsin.
DUMM, MARY E., Graduate Student, Bryn Mawr College.
DZIEMIAN, ARTHUR J., National Research Fellow in Zoology, University of Pennsylvania, School of Medicine.
EDWARDS, GEORGE A., Graduate Assistant in Biology, Tufts College.
EVANS, TITUS C., Research Assistant Professor of Radiology, State University of Iowa.
FAILLA, G., Physicist, Memorial Hospital.
FISHER, KENNETH C., Assistant Professor of Experimental Biology, University of Toronto.
FORBES, JAMES, Assistant Professor of Biology, Fordham University.
FURTH, JACOB, Associate Professor of Pathology, Cornell University Medical College.
GARREY, W. E., Professor of Physiology, Vanderbilt University, School of Medicine.
GILBERT, PERRY W., Instructor in Zoology, Cornell University.
GILMAN, LAUREN C., Johns Hopkins University.
GOODRICH, H. B., Professor of Biology, Wesleyan University.
GRAVE, BENJAMIN H., Professor of Zoology, DePauw University.
GRAVE, CASWELL, Professor of Zoology, Emeritus, Washington University.
GUDERNATSCH, FREDERICK, Visiting Professor of Biology, Washington Square College, New York University.
GUTTMAN, RITA, Instructor, Brooklyn College.
HAMBURGER, VIKTOR, Associate Professor, Washington University.
HARNLY, MORRIS H., Associate Professor, Washington Square College, New York University.
HARTMAN, FRANK A., Professor of Physiology, Ohio State University.
HARVEY, E. NEWTON, Professor of Physiology, Princeton University.

HARVEY, ETHEL B., Research Investigator, Princeton University.
HAYASHI, TERU, Graduate Assistant, University of Missouri.
HAYWOOD, CHARLOTTE, Associate Professor of Physiology, Mount Holyoke College.
HEILBRUNN, L. V., Associate Professor of Zoology, University of Pennsylvania.
HENDRICKS, ELIOTT M., University of Cincinnati.
HIBBARD, HOPE, Professor, Oberlin College.
HILL, SAMUEL E., Professor of Biology, Russell Sage College.
HOPKINS, DWIGHT L., Professor, Mundelein College.
HOWE, H. E., Editor, Industrial and Engineering Chemistry, Washington, D. C.
HUNNINEN, ARNE V., Professor of Biology, Oklahoma City University.
HUNTER, GEORGE W., Assistant Professor of Biology, Wesleyan University.
HUTCHENS, JOHN O., Johnston Scholar, Johns Hopkins University.
ILLICK, J. THERON, Associate Professor of Zoology, Syracuse University.
JACOBS, M. H., Professor of General Physiology, University of Pennsylvania.
JANOWITZ, OLGA, Instructor, Potomac School, Washington, D. C.
JENKINS, GEORGE B., Professor of Anatomy, Emeritus, George Washington University.
JONES, E. RUFFIN, JR., Professor of Biology, College of William and Mary.
KAYLOR, CORNELIUS T., Instructor in Anatomy, Syracuse University, College of Medicine.
KEMPTON, RUDOLF T., Professor of Zoology, Vassar College.
KEOSIAN, JOHN, Assistant Professor of Biology, University of Newark.
KNOWLTON, FRANK P., Professor of Physiology, Syracuse University, College of Medicine.
KOPAC, M. J., Visiting Assistant Professor of Biology, Washington Square College, New York University.
KRAHL, M. E., Research Chemist, Eli Lilly and Company.
LANCEFIELD, DONALD E., Associate Professor of Biology, Queens College.
LANSING, ALBERT I., Assistant, Indiana University.
LERNER, EDWIN M., II., Harvard University.
LIEBMANN, EMIL, Research Fellow, Tulane University.
LILLIE, FRANK R., Professor of Embryology, Emeritus, The University of Chicago.
LILLIE, RALPH S., Professor of Physiology, Emeritus, The University of Chicago.
LITTLE, E. P., Instructor, Exeter Academy.
LUCAS, ALFRED M., Associate Professor, Iowa State College.
MARKELL, EDWARD K., Teaching Assistant in Zoology, University of California.
MARSLAND, DOUGLAS A., Assistant Professor of Biology, Washington Square College, New York University.
MARTIN, WALTER E., Assistant Professor of Zoology, DePauw University.
MARVEL, ROBERT, Bartlett, New Hampshire.
MAST, S. O., Professor of Zoology, Johns Hopkins University.
MATHEWS, ALBERT P., Professor of Biochemistry, Emeritus, University of Cincinnati.
MATTOX, N. T., Assistant Professor of Zoology, Miami University.
MAYOR, JAMES W., Professor of Biology, Union College.
MCCLUNG, C. E., Professor of Zoology, Emeritus, University of Pennsylvania.
MENKIN, VALY, Assistant Professor of Pathology, Harvard University Medical School.
METZ, C. W., Head, Department of Zoology, University of Pennsylvania.
MEYERHOF, PROF. O. H., Research Professor of Biochemistry, University of Pennsylvania.
MICHAELIS, LEONOR, Member Emeritus, Rockefeller Institute.
MILLER, JAMES A., Instructor in Anatomy, University of Michigan.
MILNE, LORUS J., Associate Professor of Biology, Randolph-Macon Woman's College.
MITCHELL, PHILIP H., Professor of Biology, Brown University.

MOLNAR, GEORGE W., Instructor in Zoology, Miami University.
MOORE, WALTER G., Instructor, Loyola University of the South.
MORGAN, ISABEL M., Assistant, Rockefeller Institute.
MORGAN, LILIAN V., Pasadena, California.
MORGAN, T. H., Professor of Biology, California Institute of Technology.
MORRILL, C. V., Associate Professor of Anatomy, Cornell University Medical College.
NACHMANSOHN, DAVID, Research Fellow, Yale University, School of Medicine.
NAVEZ, ALBERT E., In Charge of Tutorial System, Milton Academy.
NORTHROP, JOHN H., Member, Rockefeller Institute for Medical Research.
O'BRIEN, JOHN P., Johns Hopkins University.
OLSON, MAGNUS, Instructor in Zoology, University of Minnesota.
OPPENHEIMER, JANE M., Instructor in Biology, Bryn Mawr College.
OSBORN, CLINTON M., Instructor in Anatomy, Ohio State University.
OSTERHOUT, W. J. V., Member Emeritus, Rockefeller Institute for Medical Research.
OSTERUD, KENNETH L., Graduate Assistant in Biology, New York University.
PACKARD, CHARLES, Assistant Professor of Zoology, Cancer Research, Columbia University.
PARKER, G. H., Professor of Zoology, Emeritus, Harvard University.
PARMENTER, CHARLES L., Professor of Zoology, University of Pennsylvania.
PARPART, ARTHUR K., Associate Professor, Princeton University.
PHELPS, LILLIAN A., Assistant Professor of Biology, Washburn College.
PICK, JOSEPH, Instructor in Anatomy, New York University, College of Medicine.
PLOUGH, HAROLD H., Professor of Biology, Amherst College.
POND, SAMUEL E., Technical Manager, Marine Biological Laboratory.
PROSSER, C. LADD, Assistant Professor of Zoology, University of Illinois.
RANKIN, JOHN S., Instructor in Biology, Amherst College.
RENSHAW, BIRDSEY, Assistant Professor, Oberlin College.
RIS, HANS, Assistant in Zoology, Columbia University.
RONA, ELIZABETH, Holder of a Carnegie Fellowship, Geophysical Laboratory, Carnegie Institution.
RUEBUSH, T. K., Instructor in Zoology, Yale University.
RUNK, B. F. D., Instructor, University of Virginia.
SANDOW, ALEXANDER, Assistant Professor of Biology, Washington Square College, New York University.
SAYLES, LEONARD P., Assistant Professor, College of the City of New York.
SCHAEFFER, A. A., Professor of Biology, Temple University.
SCHECHTER, VICTOR, Assistant Professor of Biology, College of the City of New York.
SCHOTTE, OSCAR E., Associate Professor of Biology, Amherst College.
SCOTT, ALLAN, Assistant Professor, Union College.
SCOTT, SISTER FLORENCE MARIE, Professor of Biology, Seton Hill College.
SHAPIRO, HERBERT, Instructor in Physiology, Vassar College.
SHAW, MYRTLE, Senior Bacteriologist, New York State Department of Health.
SHELDEN, FREDERICK F., Instructor in Physiology, Ohio State University.
SICHEL, ELSA KEIL, Head of the Science Department, State Normal School, Johnson, Vermont.
SICHEL, F. J. M., Assistant Professor of Physiology, University of Vermont, College of Medicine.
SLIFER, ELEANOR H., Assistant Professor, State University of Iowa.
SMITH, JAY A., Instructor in Physiology and Pharmacology, Chicago Medical School.
SPEIDEL, CARL C., Professor of Anatomy, University of Virginia.
STEINBACH, H. BURR, Assistant Professor of Zoology, Columbia University.
STERN, KURT G., Research Assistant Professor, Yale University.

STEWART, DOROTHY R., Associate Professor of Biology, Skidmore College.
STOKEY, ALMA G., Professor of Botany, Mount Holyoke College.
STUNKARD, HORACE W., Professor of Biology, New York University.
STURTEVANT, A. H., Professor, California Institute of Technology.
TASHIRO, SHIRO, Professor of Biochemistry, University of Cincinnati.
TAYLOR, WILLIAM R., Professor of Botany, University of Michigan.
TEWINKEL, LOIS E., Assistant Professor of Zoology, Smith College.
TRAGER, WILLIAM, Associate, Rockefeller Institute for Medical Research.
TROMBETTA, VIVIAN V., Instructor in Botany, Smith College.
TURNER, ABBY H., Professor of Physiology, Emeritus, Mount Holyoke College.
VELICK, SIDNEY F., Research Fellow, Yale University.
WALD, GEORGE, Faculty Instructor, Harvard University.
WALKER, ROLAND, Instructor in Biology, Rensselaer Polytechnic Institute.
WARNER, EDWARD N., Instructor in Zoology, Ohio State University.
WATERMAN, ALLYN J., Assistant Professor of Biology, Williams College.
WATTERSON, RAY L., Graduate Teaching Assistant in Zoology, University of Rochester.
WENRICH, D. H., Professor of Zoology, University of Pennsylvania.
WHITING, ANNA R., Guest Investigator, University of Pennsylvania.
WHITING, P. W., Associate Professor of Zoology, University of Pennsylvania.
WICHTERMAN, RALPH, Assistant Professor of Biology, Temple University.
WILBUR, KARL M., Research Associate, Washington Square College, New York University.
WILHELMI, RAYMOND W., Graduate Assistant, New York University.
WILLIER, BENJAMIN H., Professor of Zoology and Chairman of the Department of Biology, Johns Hopkins University.
WOLF, E. ALFRED, Associate Professor of Biology, University of Pittsburgh.
WOLF, OPAL M., Assistant Professor, Goucher College.
WOODRUFF, LORANDE L., Professor of Protozoology and Director of the Osborn Zoological Laboratory, Yale University.
WOODWARD, ALVALYN E., Assistant Professor of Zoology, University of Michigan.
WOODWARD, ARTHUR A., JR., Graduate Assistant, New York University.
WRINCH, DOROTHY, Lecturer in Chemistry, Johns Hopkins University.
YNTEMA, CHESTER L., Assistant Professor of Anatomy, Cornell University Medical College.
ZINN, DONALD J., Graduate Student, Yale University.
ZWEIFACH, BENJAMIN W., Research Associate, New York University.

Beginning Investigators

ALLEN, JOAN, Student, 1105 Park Avenue, New York City, N. Y.
ALSUP, FRED W., Graduate Student, University of Pennsylvania.
AQUILA, SISTER M., Graduate Student, Villanova College.
BENEDICT, DORA, Cohasset, Massachusetts.
BERNHEIMER, ALAN W., Graduate Student, University of Pennsylvania.
BIRMINGHAM, LLOYD, Graduate Teaching Assistant, University of Rochester.
BONGIOVANNI, ALFRED M., Investigator, Villanova College.
BURT, RICHARD L., Junior Research Fellow, Brown University.
COLE, ROGER M., Teaching Fellow, Harvard University.
COMPTON, ALFRED D., JR., Graduate Student, Yale University.
DUNCAN, GEORGE W., Halstead Fellow in Surgery, Johns Hopkins University.
ELIZABETH, SISTER MIRIAM, Associate Professor of Biology, Chestnut Hill College.
GABRIEL, MORDECAI L., Assistant in Zoology, Columbia University.
GENEVIEVE, SISTER MARY, Graduate Student, Villanova College.
GILBERT, WILLIAM J., University of Michigan.
GLANCY, ETHEL A., Tutor, Queens College.

GOLDIN, ABRAHAM, Graduate Student, Columbia University.
GRAND, C. G., Research, Washington Square College, New York University.
GREEN, JAMES WATSON, Graduate Student, University of Pennsylvania.
GROUPE, VINCENT, Graduate Student, University of Pennsylvania.
HAAS, WARD J., Undergraduate, Massachusetts Institute of Technology.
HAMILTON, PAULINE G., Research Assistant and Graduate Student, University of Pennsylvania.
HARRIS, DANIEL L., Instructor, University of Pennsylvania.
HASSETT, CHARLES, Graduate Assistant, Johns Hopkins University.
HAYES, E. R., Graduate Assistant in Anatomy, Ohio State University.
HENDLEY, CHARLES D., Assistant in Biophysics, Columbia University.
HENDRICKS, ANNE L., Medical Student, University of Cincinnati.
HENRY, RICHARD J., Student, University of Pennsylvania, School of Medicine.
HENSON, MARGARET, Teaching Fellow in Biology, Washington Square College, New York University.
HICKSON, ANNA K., Research Chemist, Eli Lilly and Company.
HINCHEY, M. CATHERINE, Graduate Student, University of Pennsylvania.
HOPKINS, MARJORIE G., Graduate Assistant, Mount Holyoke College.
HORN, ANNABELLE BROOMALL, Graduate Student, University of Pittsburgh.
HOUCK, C. RILEY, Sterry Junior Fellow, Princeton University.
HYMAN, CHESTER, Research Assistant, New York University.
KLOTZ, JOHN W., Graduate Student, University of Pittsburgh.
LEVIN, ERWIN, Student, Ohio State University.
MCNUTT, W. S., JR., Assistant in Biology, Brown University.
MELKON, BERNARD, University of Pennsylvania.
METZ, CHARLES B., Teaching Fellow in Embryology, California Institute of Technology.
MOLTER, JOHN A., Graduate Student, University of Pennsylvania.
MOOG, FLORENCE, Graduate Student, Columbia University.
MUIR, ROBERT M., Rackham Predoctoral Fellow, University of Michigan.
OESTERLE, PAUL DANIEL, Biology Instructor, Chestnut Hill College.
RECKNAGEL, RICHARD, Graduate Student, University of Pennsylvania.
ROLLASON, HERBERT D., JR., Graduate Assistant, Williams College.
SAUNDERS, GRACE, Teaching Fellow, Washington Square College, New York University.
SHELANSKI, LOUIS, Graduate Student, University of Pennsylvania.
STERN, JOSEPH R., Research Assistant, University of Toronto.
THIVY, FRANCESCA, Graduate Student, University of Michigan.
TROEDSSON, PAULINE H., Student, Columbia University.
WEISIGER, JAMES R., Graduate Fellow in Physiological Chemistry, Johns Hopkins University.
WIERCINSKI, FLOYD J., Research Assistant, University of Pennsylvania.
WILLIAMS, JOHN L., Graduate Assistant, New York University.
WILSON, WALTER L., Graduate Student, University of Pennsylvania.
ZARUDNAYA, KATYA, Student, Radcliffe College.
ZIMMERMAN, GEORGE L., Student, Swarthmore College.
ZINGHER, JOSEPH M., College of the City of New York.
ZORZOLI, ANITA, Graduate Student, Washington Square College, New York University.

Research Assistants

BAKER, LINVILLE A., Research Assistant in Physiological Chemistry, Eli Lilly and Company.
BRADFORD, AUDREY A., Student, Vassar College.
BROWNELL, KATHARINE A., Research Assistant, Ohio State University.

COHEN, IRVING, Research Assistant, New York University.
CONGER, MARTHA, New York State Department of Health.
CRAWFORD, JOHN D., Milton Academy.
DONOVAN, MARY K., Graduate Student, Villanova College.
DUBOIS, ARTHUR B., Milton Academy.
DYTCHE, MARYON, Graduate Assistant in Physiology, University of Pittsburgh.
ERLANGER, MARGARET, Instructor, West Virginia University.
FERGUSON, FREDERICK P., Teaching Assistant, University of Minnesota.
FINKEL, ASHER J., Research Assistant, The University of Chicago.
GARNER, HAROLD, Research Assistant, The University of Chicago.
GELBACK, ELIZABETH L., Assistant, Yale University.
GOLDINGER, J. M., Research Assistant, The University of Chicago.
HAGER, RUSSELL P., Research Assistant, University of Pennsylvania.
HAMILTON, HOWARD L., Research Assistant, Johns Hopkins University.
HEILBRUNN, CONSTANCE, Philadelphia, Pennsylvania.
HOHWIELER, HAROLD J., Graduate Assistant, Washington University.
JACOBS, JOYE, Assistant in Physiology, University of Maryland, School of Medicine.
JANDORF, BERNHARD J., Research Assistant, Eli Lilly and Company.
KEEFE, EUGENE L., Research Assistant, Washington University.
MARMONT, GEORGE, Research Associate in Physiology, College of Physicians and Surgeons, Columbia University.
MARTIN, ROSEMARY D. C., Demonstrator, University of Toronto.
MAURER, JANE, Yale University Medical School.
MEAD, FRANK W., Ohio State University.
MEED, MARGARET R., Graduate Student, Brown University.
MULLINS, LORIN J., Assistant in Physiology, University of Rochester.
NASH, GERRY H., Research Assistant, New York University, College of Medicine.
NELSON, GORDON H., Assistant, Milton Academy.
NETSKY, MARTIN, Medical Student, University of Pennsylvania.
ORMSBEE, RICHARD A., Laboratory Assistant, Brown University.
OSTERUD, DOROTHY W., Research Assistant, New York University.
PERKINS, PATRICIA J., Graduate Student, Uniyersity of Cincinnati.
PHILIPS, FREDERICK S., Seessel Fellow in Biology, Yale University.
RONKIN, RAPHAEL R., Graduate Student, University of California.
ROTHSTEIN, ASER, Graduate Assistant, University of Rochester.
SCHAFFEL, MILTON, Research Assistant, University of Pittsburgh.
SHAPIRO, HENRY, Williams College.
SLAUGHTER, JOSEPH C., Research Assistant, State University of Iowa.
SPIEGELMAN, S., Research Assistant, Columbia University.
SPRATT, NELSON T., JR., Research Assistant, Johns Hopkins University.
STEBBINS, ROBERT B., Assistant, Washington Square College, New York University.
SULLIVAN, PAULINE F., Teacher, Choate School, Brookline, Massachusetts.
SUTRO, PETER J., Student, Harvard University.
TRINKAUS, J. PHILIP, Cramer Fellow, Dartmouth College.
TSENG, C. K., Fellow in Botany, University of Michigan.
WARREN, ALATHIER A., Assistant, Harvard University Medical School.
WASSERMAN, EDWARD, Undergraduate Assistant, Wesleyan University.
WEAVER, MARGARET A., Graduate Student, University of Texas.

Library Readers, 1941

AUGER, CARLTON, Rockefeller Institute for Medical Research.
BAKER, GLADYS E., Instructor in Department of Plant Science, Vassar College.
BALL, ERIC G., Assistant Professor of Biochemistry, Harvard University Medical School.
BECK, L. V., Instructor in Physiology, Hahnemann Medical College.

BELDA, WALTER H., Professor, Fordham University.
BLOCH, ROBERT, Osborn Botanical Laboratory, Yale University.
BODIAN, DAVID, Assistant Professor of Anatomy, Western Reserve University Medical School.
BOWEN, EARL, Head of Department of Biology, Gettysburg College.
CASTELNUAVO, EJINA, University of Missouri.
COX, EDWARD H., Professor of Chemistry, Swarthmore College.
GATES, R. RUGGLES, Professor of Botany, University of London.
GRANICK, SAM, Assistant, Rockefeller Institute for Medical Research.
GRAY, IRVING E., Associate Professor of Zoology, Duke University.
GUREWICH, VLADIMIR, Cornell University Medical College.
HALL, THOMAS S., Instructor, Laurenceville School.
HUMM, FRANCES D., Research Fellow in Embryology, Yale University.
KREEZER, GEORGE L., Assistant Professor of Psychology, Cornell University.
LEVY, MILTON, Assistant Professor, New York University, College of Medicine.
LOEWI, OTTO, Research Professor of Pharmacology, New York University, College of Medicine.
MACKNIGHT, ROBERT H., Instructor, University of North Carolina.
MAGNUS-LEVY, ADOLF, Yale University.
OSTER, ROBERT H., Assistant Professor of Physiology, University of Maryland School of Medicine.
RABINOWITCH, EUGENE, Research Associate, Massachusetts Institute of Technology.
RAHN, HERMANN, Instructor, University of Wyoming.
REINER, JOHN M., Room 516, 303 Fourth Avenue, New York, New York.
ROTHEN, A., Associate, Rockefeller Institute for Medical Research.
SEVAG, MANASSEH G., Assistant Professor of Biochemistry, University of Pennsylvania, School of Medicine.
SHWARTZMAN, GREGORY, Head of Department of Bacteriology, Mount Sinai Hospital.
SOLBERG, ARCHIE N., Assistant Professor of Biology, University of Toledo.
STOWELL, ROBERT E., Research Assistant, Barnard Free Skin and Cancer Hospital.
YOUNG, WILLIAM C., Associate Professor of Primate Biology, Yale University.

Students, 1941

BOTANY

ABBOTT, CHARLES C., Student, Haverford College.
BAYARD, ELLEN B., University of Connecticut.
BULL, NANCY B., Student, Wellesley College.
ENZENBACHER, JEAN A., Smith College.
FRANTZ, RUTH E., Elmira College.
KATZ, ELAINE J., Student, Goucher College.
LEUCHS, AUGUSTA V. H. A., Graduate Student, Radcliffe College.
MUIR, ROBERT M., University of Michigan.
SALVIN, SAMUEL B., Assistant, Harvard University.
SMITH, FREDERICK B., Professor of Soils, University of Florida.
STANTON, CONNIE LEE, Bryn Mawr College.
STENGER, REV. FREDERICK R., Professor of Biology, St. Mary of the Lake Seminary.
THORNE, ROBERT F., Dartmouth College.
WALDRON, JACQUELINE M., Student, American University.
WILLIAMS, ROBERT H., Instructor in Botany, Cornell University.

EMBRYOLOGY

BERGSTROM, WILLIAM H., Student, Amherst College.
BIRMINGHAM, LLOYD W., University of Rochester.

Cole, Roger M., Teaching Fellow, Harvard University.
Covalla, Miriam J., Instructor, Seton Hill College.
Crumb, Cretyl, Wellesley College.
Eldred, Earl, Teaching Assistant, Northwestern University.
Gibbs, Elizabeth, Wheaton College.
Gross, John B., Student, DePauw University.
Hasse, Gordon W., Graduate Assistant, University of Illinois.
Hendricks, Anne L., University of Cincinnati.
Hollister, Patricia, Student, Sarah Lawrence College.
Hopkins, Marjorie G., Graduate Assistant, Mount Holyoke College.
Josephson, Neil, Assistant, Wesleyan University.
Karczmar, Alexander G., Columbia University.
Kezer, Leonard J., Biology Instructor, State Teachers College, Newark, N. J.
Kieffer, Richard F., Jr., Franklin and Marshall College.
Kirkpatrick, Elizabeth M., Connecticut College.
Lambie, Morris W., Student, Harvard University.
Lerner, Eleanor D., Graduate Student, Brooklyn College.
Linthicum, Anne H., Student, Goucher College.
Martin, Richard G., Harvard University.
Martin, T. Sterling, Oberlin College.
Medlicott, Mary, Graduate Assistant, Mount Holyoke College.
Morgan, Thomas W., Washington and Jefferson College.
Mothes, Arlene M., Student, Massachusetts State College.
Muchmore, William B., Oberlin College.
Plough, Irvin C., Student, Amherst College.
Power, Maxwell E., Graduate Assistant, Yale University.
Regnery, David C., Laboratory Instructor, Stanford University.
Reiner, Elliot R., Laboratory Assistant, University of Alabama.
Royle, Jane G., Graduate Student, Bryn Mawr College.
Saunders, Grace S., Teaching Fellow, Washington Square College.
Smithcors, J. Frederick, Rutgers University.
Svihla, George, Research Assistant in Zoology, University of Illinois.
Timanus, Alice L., Student, Converse College.
Wasserman, Edward, Undergraduate Assistant, Wesleyan University.
Zingher, Joseph M., College of the City of New York.

PHYSIOLOGY

Algire, Glenn H., Graduate Student, University of Maryland.
Burns, J. E., Jr., Graduate Assistant, Wesleyan University.
Coe, Frederick W., Ohio Wesleyan University.
Deyrup, Ingrith J., Barnard College.
Egan, Richard W., Research Assistant, Canisius College.
Gordon, Harold T., Teaching Fellow, Harvard University.
Green, James W., Student, Davis-Elkins College.
Gregg, John R., Undergraduate Assistant, University of Alabama.
Harrison, Robert W., Graduate Assistant, Wesleyan University.
Hartmann, J. Francis, Assistant, Cornell University.
Henry, Jane E., Gettysburg College.
Jacobs, Jack, Teaching Fellow, Washington Square College.
Keezer, William S., Indiana University.
Lein, Joseph, College of the City of New York.
Lorenz, Philip B., Swarthmore College.
Perkins, Patricia J., University of Cincinnati.
Rollason, Herbert D., Jr., Graduate Assistant, Williams College.
Rosenblum, Eugene D., Brooklyn College.

SCHEPARTZ, BERNARD, Student, Ohio Wesleyan University.
SCHLOSSER, ADELE P., Vassar College.
STERN, JOSEPH R., Research Assistant, University of Toronto.
TUTTLE, L. CONSTANCE, Graduate Assistant, Mount Holyoke College.
WEINER, HERBERT M., Student, Harvard University.
ZIMMERMAN, GEORGE L., Swarthmore College.

ZOOLOGY

ANDERSON, DORCAS J., Graduate Assistant, Purdue University.
ANDREWS, THOMAS J., Massachusetts State College.
ARAM, HARTLEY H., State University of Iowa.
BATCHELOR, WILLIAM H., Student, Harvard College.
BEARDSLEY, MARGARET, Smith College.
BERG, PHILIP W., State University of Iowa.
BRAINERD, JOHN W., Student, Harvard University.
BROWN, HENRIETTA B., Student, Tufts College.
BURKE, ROGER K., Undergraduate Assistant, Springfield College.
BYERRUM, RICHARD, Student, Wabash College.
CARPENTER, ELIZABETH, Mount Holyoke College.
COLE, LAMONT C., University of Chicago.
CORDER, HENRY R., Graduate Assistant, Wesleyan University.
CORNISH, HELEN R., Teacher of Biology, Virginia Intermont College.
CULBERSON, ARTHUR W., Williams College.
DODD, SAMUEL G., Wesleyan University.
DOLE, DOROTHY K., Assistant in Zoology, Vassar College.
GARMAN, ELIZABETH M., Student, New Jersey College for Women.
GILLETTE, ROY J., Graduate Assistant, Washington University.
GILLIGAN, CATHERINE, Teacher, Hyde Park High School.
GOFFIN, MARY F., Seton Hill College.
GROSS, LOUISE E., Student, Smith College.
HAHN, RHEA J., Student, Radcliffe College.
HARRIS, NORMAN D., Teacher of Biology, Berkshire School.
HAUSCHKA, THEODORE S., Harrison Fellow, University of Pennsylvania.
HEAPS, MARIAN E., Teacher of Biology, Pennsgrove.
HINDE, HOWARD P., Graduate Assistant, Yale University.
HUMM, DOUGLAS G., Graduate Assistant, Yale University.
KIELICH, F. RANDOLPH, Laboratory Assistant, Canisius College.
KING, ELLEN E., Student, Sarah Lawrence College.
KOHLER, CHARLES E., Rutgers University.
LUMB, ETHEL S., Graduate Student, University of Missouri.
MAHR, MERLE M., Graduate Assistant, New York University.
MEAD, ALBERT R., Assistant in Zoology, Cornell University.
MILLER, HELENA A., Student, Radcliffe College.
MINER, HOWARD D., JR., Student, Wabash College.
OSMUN, JOHN V., Graduate Student, Amherst College.
PERKINS, DAVID D., University of Rochester.
POND, SIDNEY, Wesleyan University.
POWERS, REV. WILLIAM T., Instructor in Zoology, DePaul University.
RANDALL, WALTER C., Graduate Assistant, Purdue University.
ROBERTS, BERYL J., Teacher, City of Boston—School Department.
ROBERTS, HENRY S., JR., Fellow, Duke University.
ROBERTS, WESLEY F., Graduate Assistant, Northwestern University.
ROBINSON, MARGARET, Graduate Student, Radcliffe College.
ROSS, LUCILLE J., Student, Barnard College.
SCHLECHTER, HELENA L., Wilson College.

SENYARD, JUANITA, Laboratory Assistant, Oberlin College.
TALMAGE, ROY VAN NESTE, Instructor in Zoology, University of Richmond.
TUTTLE, RUTH F., Student, Wheaton College.
WEBER, ANN M., Undergraduate Laboratory Assistant, Montclair State Teachers College.
WIEDER, HERBERT, Student, Hamilton College.
WILBER, CHARLES G., Graduate Assistant, Johns Hopkins University.
WILLIAMS, ROBERT W., Harvard University.
WILSON, MAE E., Teacher of Biology, Los Angeles City Schools.

TABULAR VIEW OF ATTENDANCE

	1937	*1938*	*1939*	*1940*	*1941*
INVESTIGATORS—Total	391	380	352	386	337
Independent	256	246	213	253	197
Under Instruction	74	53	60	62	59
Research Assistants	61	81	79	71	50
Library Readers					31
STUDENTS—Total	133	132	133	128	131
Zoology	57	54	55	55	55
Protozoology (Not given in 1941)	16	10	12	7	—
Embryology	35	34	36	34	37
Physiology	16	22	21	22	24
Botany	9	12	9	10	15
TOTAL ATTENDANCE	524	512	485	514	468
Less Persons Registered as both students and investigators	13	16	14	7	7
	511	496	471	507	461
INSTITUTIONS REPRESENTED—Total	165	151	162	148	144
By Investigators	134	125	132	112	102
By Students	79	67	72	79	72
SCHOOLS AND ACADEMIES REPRESENTED					
By Investigators	3	4	2	1	5
By Students	2	1	2	2	2
FOREIGN INSTITUTIONS REPRESENTED					
By Investigators	16	14	8	2	3
By Students	—	3	1	1	1

SUBSCRIBING AND CO-OPERATING INSTITUTIONS

1941

American University
Amherst College
Barnard College
Biological Institute, Philadelphia, Pennsylvania
Bowdoin College
Brooklyn College
Brown University
Bryn Mawr College
Canisius College
California Institute of Technology
College of Physicians and Surgeons
Columbia University
Cornell University
Cornell University Medical College
DePauw University
Duke University
Fordham University
Goucher College
Hamilton College
Harvard University
Harvard University Medical School
Indiana University
Industrial and Engineering Chemistry, of the American Chemical Society
Johns Hopkins University

Sarah Lawrence College
Eli Lilly & Co.
Loyola University of the South
Josiah Macy Jr. Foundation
Massachusetts State College
Mount Holyoke College
Mount Sinai Hospital, New York City
Mundelein College
New York State Department of Health
New York University
New York University College of Medicine
New York University Washington Square College
Northwestern University
Oberlin College
Ohio State University
Ohio Wesleyan University
Princeton University
Purdue University
Radcliffe College
Rockefeller Foundation
Rockefeller Institute for Medical Research
Russell Sage College
Rutgers University
Seton Hill College
Smith College
Springfield College
State University of Iowa
Syracuse University
Tufts College
Tulane University
Union College
University of Chicago
University of Cincinnati
University of Illinois
University of Maryland Medical School
University of Michigan
University of Missouri
University of Pennsylvania
University of Pennsylvania School of Medicine
University of Pittsburgh
University of Rochester
University of Toledo
University of Virginia
Vanderbilt University Medical School
Vassar College
Villanova College
Wabash College
Washington University
Washington University Medical School
Wellesley College
Wesleyan University
Wheaton College
Yale University
Yale University Medical School

THE FRIDAY EVENING LECTURES, 1941

Friday, June 27
DR. F. R. LILLIE ..... "How Feathers Are Made."
Friday, July 11
PROFESSOR K. S. LASHLEY ..... "The Integration of Neurology and Psychology."
Friday, July 18
DR. ERIC PONDER ..... "Red Cell Structure in the Light of Shape Transformations."
Friday, July 25
DR. DOROTHY WRINCH ..... "The Native Protein."
Friday, August 1
DR. ERNST MAYR ..... "Speciation in Birds."
Friday, August 8
DR. PHILIP B. ARMSTRONG ..... "Function in the Developing Gastrointestinal Tract of Amblystoma punctatum in Relation to Embryonic Determination and Differentiation."
Friday, August 15
DR. FRANZ SCHRADER ..... "The Mitotic Movements of Chromosomes."

Friday, August 22
Dr. Otto Meyerhof ..... "The Nature, Function and Distribution of the Phosphogens in the Animal Kingdom."

Friday, August 29
Dr. Rudolf Schoenheimer ..... "The Dynamic State of the Body Constituents."

ADDITIONAL LECTURES, 1941

Thursday, July 3
Dr. G. S. Avery ..... "Current Approaches to the Plant Hormone Problem."

Thursday, July 31
Dr. P. S. Galtsoff ..... "Seals in Alaska."

Wednesday, August 20
Dr. Henrik Dam ..... "The Biological Significance of Vitamin K."

Wednesday, August 27
Mr. George G. Lower ..... "Local Marine Life" in color.

Thursday, August 28
Dr. Milton Levy ..... "Chemical Studies of the Chick Embryo."

SHORTER SCIENTIFIC PAPERS, 1941

Tuesday, July 8
Dr. Eric G. Ball ..... "The Source of Pancreatic Juice Bicarbonate."
Mr. Arthur J. Dziemian ..... "The Permeability and the Lipid Content of the Erythrocytes in Experimental Anemia."
Dr. Rita Guttman and
Dr. K. S. Cole ..... "Electrical Rectification in Single Nerve Fibers."
Dr. Herbert Shapiro ..... "Metabolism and Fertilization in the Starfish Egg."

Tuesday, July 15
Dr. L. B. Clark ..... "A Suggested Mechanism by which the Moon Influences Reproduction in the Atlantic Palolo Worm."
Dr. Paul S. Galtsoff ..... "Accumulation of Manganese and the Sexual Cycle in Ostrea virginica."
Dr. R. M. Cable and
Dr. A. V. Hunninen ..... "Studies in the Life History of Siphodera, a Trematode Parasite of the Toadfish."
Dr. H. W. Stunkard ..... "Pathology and Immunity to Infection by Heterophyd Thematodes."

Tuesday, July 22

Dr. G. H. Parker ..... "The Melanophore System of Teleosts."

Dr. R. L. Watterson ..... "Some Aspects of Pigment Deposition in Feather Germs of Chick Embryos."

Dr. H. L. Hamilton ..... "The Influence of Hormones on the Differentiation of Melanophores in Birds."

Dr. Hermann Rahn ..... "The Distribution and Development of the Melanophore Hormone in the Pituitary of the Chick."

Tuesday, July 29

Dr. Kenneth C. Fisher ..... "The Fractionation of Cellular Respiration by Narcotics."

Dr. T. C. Evans and
Mr. J. C. Slaughter ..... "Radiosensitivity of Arbacia Sperm under Different Conditions."

Tuesday, July 29

Dr. A. M. Chase ..... "Effect of Azide on Cypridina luciferin."

Tuesday, August 5

Dr. Victor Schechter ..... "Aging Phenomena, and Factors Influencing the Longevity of Mactra Eggs."

Dr. Frederick S. Philips ..... "Comparison of Regional Respiratory Rates of the Chick Embryo During Early Stages of Development."

Dr. Mathilda M. Brooks ..... "Further Interpretations of the Effects of CO and CN on Oxidations in Living Cells."

Tuesday, August 12

Dr. William Trager ..... "Studies on Conditions Affecting the Survival *in vitro* of a Malarial Parasite."

Dr. Charles Hassett ..... "The Effect of Dyes on the Response to Light in Peranema."

Dr. J. D. Hutchens ..... "Utilization of Ammonia by Chilomonas paramoecium."

Miss Virginia Dewey and
Dr. G. W. Kidder ..... "The Possibility of Thiamine Synthesis by Ciliates."

Tuesday, August 19

Dr. David Nachmansohn ..... "Electrical Potential and Activity of Choline Esterase in Nerves."

Dr. Albert Claude ..... "Chemical Composition of Mitochondria and Secretory Granules."

Dr. Dorothy Wrinch ..... "Native Proteins and the Structure of Cytoplasm."

GENERAL SCIENTIFIC MEETINGS, 1941

Tuesday, August 26

DR. CASWELL GRAVE ..... I. "Further Studies of Metamorphosis of Ascidian Larvae."
II. "The 'Eye Spot' and Light Responses of the Larva of Cynthia partita."

MR. LLOYD BIRMINGHAM ..... "Regeneration in the Early Zooids of Amaroucium constellatum."

DR. IVOR CORNMAN ..... "Characteristics of the Acceleration of Arbacia Egg Cleavage in Hypotonic Seawater."

DR. ETHEL BROWNE HARVEY ..... "Material Inheritance in Echinoderm Hybrids."

DR. E. S. GUZMAN BARRON AND
DR. J. M. GOLDINGER ..... "Intermediary Carbohydrate Metabolism of Sperm and Eggs of Arbacia before and after Fertilization."
tion."

MR. J. D. CRAWFORD,
MISS D. BENEDICT,
MR. A. B. DUBOIS AND
DR. A. E. NAVEZ ..... "On Contraction of the Venus Heart."

DR. ALFRED M. LUCAS AND
MR. JAMES SNEDECOR ..... "Co-ordination of Ciliary Movement in the Modiolus Gill."

DR. LORUS J. MILNE ..... "Preparing an Animated Diagram of Somatic Mitosis."

DR. E. NEWTON HARVEY ..... "Stimulation by Intense Flashes of Ultra Violet Light."

DR. T. C. EVANS,
DR. G. FAILLA,
MR. J. C. SLAUGHTER AND
DR. E. P. LITTLE ..... "Influence of the Medium on the Radiosensitivity of Arbacia Sperm."

DR. C. LADD PROSSER AND
MR. G. L. ZIMMERMAN ..... "Comparative Pharmacology of Myogenic and Neurogenic Hearts."

DR. LOIS E. TEWINKEL ..... "Structures Concerned with Yolk Absorption in the Dogfish, Squalus acanthias."

DR. W. H. F. ADDISON ..... "The Distribution of Elastic Tissue in the Arterial Pathway to the Carotid Bodies in the Dog."

DR. RICHARD G. ABELL AND
DR. IRVINE H. PAGE ..... "Behavior of the Arterioles in Hypertensive Rabbits, and in Normal Rabbits Following Injection of Angiotinin."

Wednesday, August 27

Dr. M. H. Jacobs and
Dr. Dorothy R. Stewart ..... "Catalysis of Ionic Exchanges by Bicarbonates."

Dr. Dorothy R. Stewart and
Dr. M. H. Jacobs ..... "The Role of Carbonic Anhydrase in the Catalysis of Ionic Exchanges by Bicarbonates."

Mr. Martin G. Netsky and
Dr. M. H. Jacobs ..... "Some Effects of Desoxycorticosterone and Related Compounds on the Mammalian Red Cell."

Dr. Herbert Shapiro and
Dr. Hugh Davson ..... "Permeability of the Arbacia Egg to Potassium."

Dr. A. K. Parpart ..... "Lipo-protein Complexes in Arbacia Eggs."

Dr. S. E. Hill ..... "Relation Between the Action Potential and Protoplasmic Streaming in Chara and Nitella."

Dr. Aurin M. Chase ..... "Observations on Luminescence in Mnemiopsis."

PAPERS READ BY TITLE

Mr. Fred W. Alsup ..... "Photodynamic Studies on Arbacia Eggs."

Dr. T. C. Evans ..... "The Effect of Roentgen Radiation on the Jelly of the Nereis Zygote."

Dr. R. Ruggles Gates ..... "Tests of Nucleoli and Cytoplasmic Granules in Marine Eggs."

Dr. Russell P. Hager ..... "Sex-linkage of Stubby (sb) in Habrobracon."

Mr. E. R. Hayes ..... "The Elasmobranch Interrenal; a Preliminary Note. The Interrenal Body of Alopias vulpinus (Bonnaterre)."

Dr. Dwight L. Hopkins ..... "The Cytology of Amoeba Verrucosa."

Dr. George W. Hunter, III and
Mr. Edward Wasserman ..... "Observations on the Melanophore Control of the Cunner Tautogolabrus adspersus (Walbaum)."

Miss Florence Moog ..... "The Influence of Temperature on Reconstitution in Tubularia."

Dr. Clinton M. Osborn ..... "Factors Influencing the Pigmentation of Regenerating Scales on the Ventral Surface of the Summer Flounder."

Dr. G. H. Parker ..... "Hypersensitization of Catfish Melanophores to Adrenaline by Denervation."

Dr. Leonard P. Sayles ..... "Implants Consisting of Young Buds, Formed in Anterior Regeneration in Clymenella, Plus the Nerve Cord of the Adjacent Old Part."

Dr. A. A. Schaeffer ..... "Chaos Nobilis Penard in Permanent Culture."

Dr. Allyn Waterman ..... "Ectodermization of the Larva of Arbacia."

Dr. Ralph Wichterman ..... "Studies on Zoochlorella-free Paramecium Bursaria."

Mr. Floyd J. Wiercinski ..... "An Experimental Study of Intracellular pH in the Arbacia Egg."

Dr. E. Alfred Wolf,
Miss Maryon Dytche and
Mr. Milton Schaffel ..... "Heat Produced by Respiring Whole Blood of Tautoga onitis and Mustelus canis."

Dr. C. L. Yntema ..... "Effect of Differences Between Stages of Donor and Host Upon Induction of Auditory Vesicle from Foreign Ectoderm in the Salamander Embryo."

DEMONSTRATIONS

Wednesday, August 27

Mr. L. F. Boss and
Dr. M. H. Jacobs ..... "Stabilized Source of Current for Lamps and Other Purposes."

Dr. E. R. Clark and
Mrs. Eleanor Linton Clark .... "Behavior of Giant Cells as Observed in the Living Mammal."

Dr. E. N. Harvey and
Dr. F. J. M. Sichel .....
1. "Apparatus for Intense Flashes of Ultra Violet Light, and the Killing of Small Organisms."
2. "Apparatus for High Speed Photography with the Microscope."

Dr. Kurt G. Stern ..... "An Air-driven High-speed Centrifuge for Optical Observations."

Dr. Ivor Cornman ..... "Disruption of Mitosis in Colchicum by Colchicine."

Dr. Sears Crowell .....
1. "Nematostella, a Simple Anemone, Suitable for Laboratory Work in General Zoology."
2. "The Nematosomes of Nematostella."

Dr. John Keosian .....
1. "Apparatus of Simple Construction for Use in Microchemical Work."
2. "A Spot Test Method for the Quantitative Determination of Magnesium in Tenths of a Microgram."

DR. ELEANOR H. SLIFER ....."A Mutant Drosophila Melanogaster with Extra Sex Combs."

DR. LOIS E. TEWINKEL ....."Structures Concerned with Yolk Absorption in the Dogfish."

DR. ERIC LOEWENSTEIN ....."Demonstration of Fluorophotometer, and Discussion of Fluorometric Methods of Determining Biological Substances."

DR. LAURENCE IRVING,
DR. P. F. SCHOLANDER,
MR. C. LLOYD CLAFF,
MR. GEORGE EDWARDS AND
MR. NIELS HAUGAARD .:.....Section A

Sensitive volumetric apparatus devised by Dr. P. F. Scholander, as used in the continuous measurement of respiration and in the measured delivery of small quantities of liquid.

1. "A Respirometer with Mechanical Circulation of Air, Sensitive to 0.3 mm^3., Used to Observe Long Continuous Measurements of O_2 Consumption in Water of Animals of Approximately ½ Gram Body Weight." *Edwards.*
2. "A Respirometer Similar in Construction to the Above, But Lacking a Mechanical Circulation, Sensitive to 0.15 mm^3., Used to Observe Long Continuous Measurements of the O_2 Consumption of Protozoa (5,000–10,000 Organisms)."
3. "A Micrometer Calibrating Burette, Sensitive to 0.3 mm^3., Used for the Delivery of Small Quantities of Liquid." *Claff and Edwards.*
4. "A Micro-micrometer Calibrating Burette, Sensitive to 0.02 mm^3., Used for the Delivery of Minute Quantities of Liquid." *Claff.*
5. "The Small Gear Pump Used for the Circulation of Air in the Respirometer, Devised by Dr. Scholander, Constructed Under the Direction of C. Lloyd Claff by Mr. Carl A. Moeller." *Claff.*

Section B

"A System Suitable for Aquatic Animals, Sensitive to 0.02 cc., Used to Measure the O_2 Consumption of Fishes of 20–200 Grams Body Weight at 10 Minute Intervals for Periods Lasting for 12 to 24 Hours." *Haugaard.*
(The glassblowing was done by Mr. J. D. Graham.)

9. MEMBERS OF THE CORPORATION, 1941

1. Life Members

Allis, Mr. E. P., Jr., Palais Carnoles, Menton, France.
Andrews, Mrs. Gwendolen Foulke, Baltimore, Maryland.
Beckwith, Dr. Cora J., Vassar College, Poughkeepsie, New York.
Billings, Mr. R. C., 66 Franklin Street, Boston, Massachusetts.
Calvert, Dr. Philip P., University of Pennsylvania, Philadelphia, Pennsylvania.
Conklin, Prof. Edwin G., Princeton University, Princeton, New Jersey.
Evans, Mrs. Glendower, 12 Otis Place, Boston, Massachusetts.
Foot, Miss Katherine, Care of Morgan Harjes Cie, Paris, France.
Gardiner, Mrs. E. G., Woods Hole, Massachusetts.
Jackson, Mr. Chas. C., 24 Congress Street, Boston, Massachusetts.
Jackson, Miss M. C., 88 Marlboro Street, Boston, Massachusetts.
King, Mr. Chas. A.
Kingsbury, Prof. B. F., Cornell University, Ithaca, New York.
Lewis, Prof. W. H., Johns Hopkins University, Baltimore, Maryland.
Lowell, Mr. A. L., 17 Quincy Street, Cambridge, Massachusetts.
Means, Dr. J. H., 15 Chestnut Street, Boston, Massachusetts.
Moore, Dr. George T., Missouri Botanical Gardens, St. Louis, Missouri.
Moore, Dr. J. Percy, University of Pennsylvania, Philadelphia, Pa.
Morgan, Mr. J. Pierpont, Jr., Wall and Broad Streets, New York City, New York.
Morgan, Mrs. T. H., Pasadena, California.
Morgan, Prof. T. H., Director of Biological Laboratory, California Institute of Technology, Pasadena, California.
Morrill, Dr. A. D., Hamilton College, Clinton, New York.
Noyes, Miss Eva J.
Porter, Dr. H. C., University of Pennsylvania, Philadelphia, Pennsylvania.

Scott, Dr. Ernest L., Columbia University, New York City, N. Y.
Sears, Dr. Henry F., 86 Beacon Street, Boston, Massachusetts.
Shedd, Mr. E. A.
Thorndike, Dr. Edward L., Teachers College, Columbia University, New York City, New York.
Treadwell, Prof. A. L., Vassar College, Poughkeepsie, New York.
Trelease, Prof. William, University of Illinois, Urbana, Illinois.
Waite, Prof. F. C., 144 Locust Street, Dover, New Hampshire.
Wallace, Louise B., 359 Lytton Avenue, Palo Alto, California.

2. Regular Members

Abramowitz, Dr. Alexander A., Biological Laboratories, Harvard University, Cambridge, Massachusetts.
Adams, Dr. A. Elizabeth, Mount Holyoke College, South Hadley, Massachusetts.
Addison, Dr. W. H. F., University of Pennsylvania Medical School, Philadelphia, Pennsylvania.
Adolph, Dr. Edward F., University of Rochester Medical School, Rochester, New York.
Albaum, Dr. Harry G., 3115 Avenue I, Brooklyn, New York.
Allee, Dr. W. C., The University of Chicago, Chicago, Illinois.
Amberson, Dr. William R., Department of Physiology, University of Maryland, School of Medicine, Lombard and Greene Streets, Baltimore, Maryland.
Anderson, Dr. Rubert S., Memorial Hospital, 444 East 68th Street, New York City, New York.
Angerer, Dr. Clifford A., Department of Physiology, Ohio State University, Columbus, Ohio.
Armstrong, Dr. Philip B., College of Medicine, Syracuse University, Syracuse, New York.
Austin, Dr. Mary L., Wellesley College, Wellesley, Massachusetts.
Baitsell, Dr. George A., Yale University, New Haven, Connecticut.
Baker, Dr. H. B., Zoological Laboratory, University of Pennsylvania, Philadelphia, Pennsylvania.
Ballard, Dr. William W., Dartmouth College, Hanover, New Hampshire.
Ballentine, Dr. Robert, Rockefeller Institute, 66th Street and York Avenue, New York City, New York.
Ball, Dr. Eric G., Department of Biological Chemistry, Harvard University Medical School, Boston, Massachusetts.
Bard, Prof. Philip, Johns Hopkins Medical School, Baltimore, Maryland.

BARRON, DR. E. S. GUZMAN, Department of Medicine, The University of Chicago, Chicago, Illinois.

BARTH, DR. L. G., Department of Zoology, Columbia University, New York City, New York.

BEADLE, DR. G. W., School of Biological Sciences, Stanford University, California.

BEAMS, DR. HAROLD W., Department of Zoology, State University of Iowa, Iowa City, Iowa.

BEHRE, DR. ELINOR H., Louisiana State University, Baton Rouge, Louisiana.

BIGELOW, DR. H. B., Museum of Comparative Zoology, Cambridge, Massachusetts.

BIGELOW, PROF. R. P., Massachusetts Institute of Technology, Cambridge, Massachusetts.

BINFORD, PROF. RAYMOND, Buck Creek Camp, Marion, North Carolina.

BISSONNETTE, DR. T. HUME, Trinity College, Hartford, Connecticut.

BLANCHARD, PROF. KENNETH C., Washington Square College, New York University, New York City, New York.

BODINE, DR. J. H., Department of Zoology, State University of Iowa, Iowa City, Iowa.

BORING, DR. ALICE M., Yenching University, Peking, China.

BRADLEY, PROF. HAROLD C., University of Wisconsin, Madison, Wisconsin.

BRONFENBRENNER, DR. JACQUES J., Department of Bacteriology, Washington University Medical School, St. Louis, Missouri.

BROOKS, DR. MATILDA M., 630 Woodmont Avenue, Berkeley, California.

BROOKS, DR. S. C., University of California, Berkeley, California.

BROWN, DR. DUGALD E. S., New York University, College of Medicine, New York City, New York.

BROWN, DR. FRANK A., JR., Department of Zoology, Northwestern University, Evanston, Illinois.

BUCKINGHAM, MISS EDITH N., Sudbury, Massachusetts.

BUCK, DR. JOHN B., Department of Zoology, University of Rochester, Rochester, New York.

BUDINGTON, PROF. R. A., Winter Park, Florida.

BULLINGTON, DR. W. E., Randolph-Macon College, Ashland, Virginia.

BUMPUS, PROF. H. C., Duxbury, Massachusetts.

BYRNES, DR. ESTHER F., 1803 North Camac Street, Philadelphia, Pennsylvania.

CALKINS, PROF. GARY N., Columbia University, New York City, New York.

CANNAN, PROF. R. K., New York University College of Medicine, 477 First Avenue, New York City, New York.

CARLSON, PROF. A. J., Department of Physiology, The University of Chicago, Chicago, Illinois.

CAROTHERS, DR. E. ELEANOR, 134 Avenue C. East, Kingman, Kansas.

CARPENTER, DR. RUSSELL L., Tufts College, Tufts College, Massachusetts.

CARROLL, PROF. MITCHEL, Franklin and Marshall College, Lancaster, Pennsylvania.

CARVER, PROF. GAIL L., Mercer University, Macon, Georgia.

CATTELL, DR. MCKEEN, Cornell University Medical College, 1300 York Avenue, New York City, New York.

CATTELL, PROF. J. MCKEEN, Garrison-on-Hudson, New York.

CATTELL, MR. WARE, Garrison-on-Hudson, New York.

CHAMBERS, DR. ROBERT, Washington Square College, New York University, Washington Square, New York City, New York.

CHASE, DR. AURIN M., Princeton University, Princeton, New Jersey.

CHENEY, DR. RALPH H., Biology Department, Long Island University, Brooklyn, New York.

CHIDESTER, PROF. F. E., Auburndale, Massachusetts.

CHILD, PROF. C. M., Jordan Hall, Stanford University, California.

CHURNEY, DR. LEON, 155 Powell Lane, Upper Darby, Pennsylvania.

CLAFF, MR. C. LLOYD, Department of Biology, Brown University, Providence, Rhode Island.

CLARK, PROF. E. R., University of Pennsylvania Medical School, Philadelphia, Pennsylvania.

CLARK, DR. LEONARD B., Department of Biology, Union College, Schenectady, New York.

CLELAND, PROF. RALPH E., Indiana University, Bloomington, Indiana.

CLOWES, DR. G. H. A., Eli Lilly and Company, Indianapolis, Indiana.

COE, PROF. W. R., Yale University, New Haven, Connecticut.

COHN, DR. EDWIN J., 183 Brattle Street, Cambridge, Massachusetts.

COLE, DR. ELBERT C., Department of Biology, Williams College, Williamstown, Massachusetts.

COLE, DR. KENNETH S., College of Physicians and Surgeons, Columbia University, 630 West 168th Street, New York City, New York.

COLE, DR. LEON J., College of Agriculture, Madison, Wisconsin.

COLLETT, DR. MARY E., Western Reserve University, Cleveland, Ohio.

COLTON, PROF. N. S., Box 601, Flagstaff, Arizona.

COOPER, DR. KENNETH W., Department of Biology, Princeton University, Princeton, New Jersey.

COPELAND, PROF. MANTON, Bowdoin College, Brunswick, Maine.

COSTELLO, DR. DONALD P., School of Biology, Stanford University, California.

COSTELLO, DR. HELEN MILLER, School of Biology, Stanford University, California.

COWDRY, DR. E. V., Washington University, St. Louis, Missouri.

CRAMPTON, PROF. H. E., Barnard College, Columbia University, New York City, New York.

CRANE, MRS. C. R., Woods Hole, Massachusetts.

CROWELL, DR. P. S., JR., Department of Zoology, Miami University, Oxford, Ohio.

CURTIS, DR. MAYNIE R., 377 Dexter Trail, Mason, Michigan.

CURTIS, PROF. W. C., University of Missouri, Columbia, Missouri.

DAN, DR. KATSUMA, Misaki Biological Station, Misaki, Japan.

DAVIS, DR. DONALD W., College of William and Mary, Williamsburg, Virginia.

DAWSON, DR. A. B., Harvard University, Cambridge, Massachusetts.

DAWSON, DR. J. A., The College of the City of New York, New York City, New York.

DEDERER, DR. PAULINE H., Connecticut College, New London, Connecticut.

DILLER, DR. WILLIAM F., 1016 South 45th Street, Philadelphia, Pennsylvania.

DODDS, PROF. G. S., Medical School, University of West Virginia, Morgantown, West Virginia.

DOLLEY, PROF. WILLIAM L., University of Buffalo, Buffalo, New York.

DONALDSON, DR. JOHN C., University of Pittsburgh, School of Medicine, Pittsburgh, Pennsylvania.

DUBOIS, DR. EUGENE F., Cornell University Medical College, 1300 York Avenue, New York City, New York.

DUGGAR, DR. BENJAMIN M., University of Wisconsin, Madison, Wisconsin.

DUNGAY, DR. NEIL S., Carleton College, Northfield, Minnesota.

DURYEE, DR. WILLIAM R., Department of Biology, Washington Square College, New York University, New York City, New York.

EDWARDS, DR. D. J., Cornell University Medical College, 1300 York Avenue, New York City, New York.

ELLIS, DR. F. W., Monson, Massachusetts.

EVANS, DR. TITUS C., 723 Kirkwood, Iowa City, Iowa.

FAILLA, DR. G., Memorial Hospital, 444 E. 68th Street, New York City, New York.

FAURÉ-FREMIET, PROF. EMMANUEL, Collège de France, Paris, France.

FERGUSON, DR. JAMES K. W., Department of Pharmacology, University of Toronto, Ontario, Canada.

FIGGE, DR. F. H. J., Yale University, School of Medicine, New Haven, Connecticut.

FISCHER, DR. ERNST, Department of Physiology, Medical College of Virginia, Richmond, Virginia.

FISHER, DR. JEANNE M., Department of Biochemistry, University of Toronto, Toronto, Canada.

FISHER, DR. KENNETH C., Department of Biology, University of Toronto, Toronto, Canada.

FLEISHER, DR. MOYER S., 20 North Kingshighway, St. Louis, Missouri.

FORBES, DR. ALEXANDER, Harvard University Medical School, Boston, Massachusetts.

FRISCH, DR. JOHN A., Canisius College, Buffalo, New York.

FRY, DR. HENRY J., Old Danbury Road, Westport, Connecticut.

FURTH, DR. JACOB, Cornell University Medical College, 1300 York Avenue, New York City, New York.

GAGE, PROF. S. H., Cornell University, Ithaca, New York.

GALTSOFF, DR. PAUL S., 420 Cumberland Avenue, Somerset, Chevy Chase, Maryland.

GARREY, PROF. W. E., Vanderbilt University Medical School, Nashville, Tennessee.

GEISER, DR. S. W., Southern Methodist University, Dallas, Texas.

GERARD, PROF. R. W., The University of Chicago, Chicago, Illinois.

GLASER, PROF. O. C., Amherst College, Amherst, Massachusetts.

GODDARD, DR. D. R., Department of Botany, University of Rochester, Rochester, New York.

GOLDFORB, PROF. A. J., College of the City of New York, Convent Avenue and 139th Street, New York City, New York.

GOODRICH, PROF. H. B., Wesleyan University, Middletown, Connecticut.

GOTTSCHALL, DR. GERTRUDE Y., Enzma Research Laboratory, U. S. Department of Agriculture, Washington, D. C.

GRAHAM, DR. J. Y., University of Alabama, University, Alabama.

GRAND, CONSTANTINE G., Biology Department, Washington Square College, New York University, Washington Square, New York, New York.

GRAVE, PROF. B. H., DePauw University, Greencastle, Indiana.

GRAVE, PROF. CASWELL, Washington University, St. Louis, Missouri.

GRAY, PROF. IRVING E., Duke University, Durham, North Carolina.

GREGORY, DR. LOUISE H., Barnard College, Columbia University, New York City, New York.

GUDERNATSCH, J. FREDRICK, New York University, 100 Washington Square, New York City, New York.

GUTHRIE, DR. MARY J., University of Missouri, Columbia, Missouri.

GUYER, PROF. M. F., University of Wisconsin, Madison, Wisconsin.

HADLEY, DR. CHARLES E., State Teachers' College, Montclair, New Jersey.

HAGUE, DR. FLORENCE, Sweet Briar College, Sweet Briar, Virginia.

HALL, PROF. FRANK G., Duke University, Durham, North Carolina.

HAMBURGER, DR. VIKTOR, Department of Zoology, Washington University, St. Louis, Missouri.

HANCE, DR. ROBERT T., Department of Biology, Duquesne University, Pittsburgh, Pennsylvania.

HARGITT, PROF. GEORGE T., Department of Zoology, Duke University, Durham, North Carolina.

HARMAN, DR. MARY T., Kansas State Agricultural College, Manhattan, Kansas.

HARNLY, DR. MORRIS H., Washington Square College, New York University, New York City, New York.

HARPER, PROF. R. A., Columbia University, New York City, New York.

HARRISON, PROF. Ross G., Yale University, New Haven, Connecticut.

HARTLINE, DR. H. KEFFER, Cornell University Medical College, 1300 York Avenue, New York City, New York.

HARTMAN, DR. FRANK A., Hamilton Hall, Ohio State University, Columbus, Ohio.

HARVEY, DR. E. NEWTON, Guyot Hall, Princeton University, Princeton, New Jersey.

HARVEY, DR. ETHEL BROWNE, 48 Cleveland Lane, Princeton, New Jersey.

HAYDEN, DR. MARGARET A., Wellesley College, Wellesley, Massachusetts.

HAYES, DR. FREDERICK R., Zoological Laboratory, Dalhousie University, Halifax, Nova Scotia.

HAYWOOD, DR. CHARLOTTE, Mount Holyoke College, South Hadley, Massachusetts.

HAZEN, DR. T. E., Barnard College, Columbia University, New York City, New York.

HECHT, DR. SELIG, Columbia University, New York City, New York.

HEILBRUNN, DR. L. V., Department of Zoology, University of Pennsylvania, Philadelphia, Pennsylvania.

HENDEE, DR. ESTHER CRISSEY, Russell Sage College, Troy, New York.

HENSHAW, DR. PAUL S., National Cancer Institute, Bethesda, Maryland.

HESS, PROF. WALTER N., Hamilton College, Clinton, New York.
HIBBARD, DR. HOPE, Department of Zoology, Oberlin College, Oberlin, Ohio.
HILL, DR. SAMUEL E., Department of Biology, Russell Sage College, Troy, New York.
HINRICHS, DR. MARIE, Department of Physiology and Health Education, South Illinois Normal University, Carbondale, Illinois.
HISAW, DR. F. L., Harvard University, Cambridge, Massachusetts.
HOADLEY, DR. LEIGH, Harvard University, Cambridge, Massachusetts.
HÖBER, DR. RUDOLF, University of Pennsylvania, Philadelphia, Pennsylvania.
HODGE, DR. CHARLES, IV, Temple University, Department of Zoology, Philadelphia, Pennsylvania.
HOGUE, DR. MARY J., University of Pennsylvania Medical School, Philadelphia, Pennsylvania.
HOLLAENDER, DR. ALEXANDER, c/o National Institute of Health, Laboratory of Industrial Hygiene, Bethesda, Maryland.
HOOKER, PROF. DAVENPORT, University of Pittsburgh, School of Medicine, Department of Anatomy, Pittsburgh, Pennsylvania.
HOPKINS, DR. DWIGHT L., Mundelein College, 6363 Sheridan Road, Chicago, Illinois.
HOPKINS, DR. HOYT S., New York University, College of Dentistry, New York City, New York.
HOWE, DR. H. E., 1155 16th St., N.W., American Chemical Society Bldg., Washington, D. C.
HOWLAND, DR. RUTH B., Washington Square College, New York University, Washington Square East, New York City, New York.
HOYT, DR. WILLIAM D., Washington and Lee University, Lexington, Virginia.
HYMAN, DR. LIBBIE H., American Museum of Natural History, New York City, New York.
IRVING, PROF. LAURENCE, Swarthmore College, Swarthmore, Pennsylvania.
ISELIN, MR. COLUMBUS O'D., Woods Hole, Massachusetts.
JACOBS, PROF. MERKEL H., School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.
JENKINS, DR. GEORGE B., 30 Gallatin Street, N. W., Washington, D. C.
JENNINGS, PROF. H. S., Department of Zoology, University of California, Los Angeles, California.
JEWETT, PROF. J. R., 44 Francis Avenue, Cambridge, Massachusetts.
JOHLIN, DR. J. M., Vanderbilt University Medical School, Nashville, Tennessee.

JONES, DR. E. RUFFIN, JR., College of William and Mary, Williamsburg, Virginia.

JUST, PROF. E. E., Howard University, Washington, D. C.

KAUFMANN, PROF. B. P., Carnegie Institution, Cold Spring Harbor, Long Island, New York.

KEMPTON, PROF. RUDOLF T., Vassar College, Poughkeepsie, New York.

KIDDER, DR. GEORGE W., Brown University, Providence, Rhode Island.

KILLE, DR. FRANK R., Swarthmore College, Swarthmore, Pennsylvania

KINDRED, DR. J. E., University of Virginia, Charlottesville, Virginia.

KING, DR. HELEN D., Wistar Institute of Anatomy and Biology, 36th Street and Woodland Avenue, Philadelphia, Pennsylvania.

KING, DR. ROBERT L., State University of Iowa, Iowa City, Iowa.

KNOWLTON, PROF. F. P., Syracuse University, Syracuse, New York.

KOPAC, DR. M. J., Washington Square College, New York University, New York City, New York.

KORR, DR. I. M., Department of Physiology, New York University, College of Medicine, 477 First Avenue, New York City, New York.

KRAHL, DR. M. E., Lilly Research Laboratories, Indianapolis, Indiana.

KRIEG, DR. WENDELL J. S., New York University, College of Medicine, 477 First Avenue, New York City, New York.

LANCEFIELD, DR. D. E., Queens College, Flushing, New York.

LANCEFIELD, DR. REBECCA C., Rockefeller Institute, 66th Street and York Avenue, New York City, New York.

LANGE, DR. MATHILDE M., Wheaton College, Norton, Massachusetts.

LEWIS, PROF. I. F., University of Virginia, Charlottesville, Virginia.

LILLIE, PROF. FRANK R., The University of Chicago, Chicago, Illinois.

LILLIE, PROF. RALPH S., The University of Chicago, Chicago, Illinois.

LOEB, PROF. LEO, Washington University Medical School, St. Louis, Missouri.

LOEWI, PROF. OTTO, 155 East 93rd Street, New York City, New York.

LOWTHER, MRS. FLORENCE DEL., Barnard College, Columbia University, New York City, New York.

LUCAS, DR. ALFRED M., Zoological Laboratory, Iowa State College, Ames, Iowa.

LUCAS, DR. MIRIAM SCOTT, Department of Zoology, Iowa State College, Ames, Iowa.

LUCKÉ, PROF. BALDUIN, University of Pennsylvania, Philadelphia, Pennsylvania.

LYNCH, DR. CLARA J., Rockefeller Institute, 66th Street and York Avenue, New York City, New York.

LYNCH, DR. RUTH STOCKING, Maryland State Teachers College, Towson, Maryland.

LYNN, DR. WILLIAM G., Department of Zoology, Johns Hopkins University, Baltimore, Maryland.

MACDOUGALL, DR. MARY S., Agnes Scott College, Decatur, Georgia.

MACLENNAN, DR. RONALD F., 1588 South Cedar Avenue, Oberlin, Ohio.

MACNAUGHT, MR. FRANK M., Marine Biological Laboratory, Woods Hole, Massachusetts.

MCCLUNG, PROF. C. E., University of Illinois, Urbana, Illinois.

MCCOUCH, DR. MARGARET SUMWALT, University of Pennsylvania Medical School, Philadelphia, Pa.

MCGREGOR, DR. J. H., Columbia University, New York City, New York.

MACKLIN, DR. CHARLES C., School of Medicine, University of Western Ontario, London, Canada.

MAGRUDER, DR. SAMUEL R., Department of Anatomy, Tufts Medical School, Boston, Massachusetts.

MALONE, PROF. E. F., College of Medicine, University of Cincinnati, Department of Anatomy, Cincinnati, Ohio.

MANWELL, DR. REGINALD D., Syracuse University, Syracuse, New York.

MARSLAND, DR. DOUGLAS A., Washington Square College, New York University, New York City, New York.

MARTIN, PROF. E. A., Department of Biology, Brooklyn College, Bedford Avenue and Avenue H, Brooklyn, New York.

MAST, PROF. S. O., Johns Hopkins University, Baltimore, Maryland.

MATHEWS, PROF. A. P., University of Cincinnati, Cincinnati, Ohio.

MATTHEWS, DR. SAMUEL A., Thompson Biological Laboratory, Williams College, Williamstown, Massachusetts.

MAVOR, PROF. JAMES W., Union College, Schenectady, New York.

MAZIA, DR. DANIEL, Department of Zoology, University of Missouri, Columbia, Missouri.

MEDES, DR. GRACE, Lankenau Research Institute, Philadelphia, Pennsylvania.

MEIGS, MRS. E. B., 1736 M Street, N. W., Washington, D. C.

MENKIN, DR. VALY, Harvard Medical School, Boston, Massachusetts.

METZ, PROF. CHARLES W., University of Pennsylvania, Philadelphia, Pennsylvania.

MICHAELIS, DR. LEONOR, Rockefeller Institute, 66th Street and York Avenue, New York City, New York.

MILLER, DR. J. A., Department of Anatomy, University of Michigan, Ann Arbor, Michigan.

MITCHELL, DR. PHILIP H., Brown University, Providence, Rhode Island.

MOORE, DR. CARL R., The University of Chicago, Chicago, Illinois.
MORGAN, DR. ISABEL M., Rockefeller Institute, York Avenue at 66th Street, New York City, New York.
MORGULIS, DR. SERGIUS, University of Nebraska, Omaha, Nebraska.
MORRILL, PROF. C. V., Cornell University Medical College, 1300 York Avenue, New York City, New York.
MOSER, DR. FLOYD, Department of Biology, University of Alabama, University, Alabama.
NAVEZ, DR. ALBERT E., Department of Biology, Milton Academy, Milton, Massachusetts.
NEWMAN, PROF. H. H., 1951 Edgewater Drive, Clearwater, Florida.
NICHOLS, DR. M. LOUISE, Rosemont, Pennsylvania.
NONIDEZ, DR. JOSÉ F., Cornell University Medical College, 1300 York Avenue, New York City, New York.
NORTHROP, DR. JOHN H., The Rockefeller Institute, Princeton, New Jersey.
OKKELBERG, DR. PETER, Department of Zoology, University of Michigan, Ann Arbor, Michigan.
OPPENHEIMER, DR. JANE M., Department of Biology, Bryn Mawr College, Bryn Mawr, Pennsylvania.
OSBURN, PROF. R. C., Ohio State University, Columbus, Ohio.
OSTERHOUT, PROF. W. J. V., Rockefeller Institute, 66th Street and York Avenue, New York City, New York.
OSTERHOUT, MRS. MARIAN IRWIN, Rockefeller Institute, 66th Street and York Avenue, New York City, New York.
PACKARD, DR. CHARLES, Columbia University, Institute of Cancer Research, 630 West 168th Street, New York City, New York.
PAGE, DR. IRVINE H., Lilly Laboratory Clinical Research, Indianapolis City Hospital, Indianapolis, Indiana.
PAPPENHEIMER, DR. A. M., Columbia University, New York City, New York.
PARKER, PROF. G. H., Harvard University, Cambridge, Massachusetts.
PARMENTER, DR. C. L., Department of Zoology, University of Pennsylvania, Philadelphia, Pennsylvania.
PARPART, DR. ARTHUR K., Princeton University, Princeton, New Jersey.
PATTEN, DR. BRADLEY M., University of Michigan Medical School, Ann Arbor, Michigan.
PAYNE, PROF. F., University of Indiana, Bloomington, Indiana.
PEEBLES, PROF. FLORENCE, Chapman College, Los Angeles, California.
PINNEY, DR. MARY E., Milwaukee-Downer College, Milwaukee, Wisconsin.

PLOUGH, PROF. HAROLD H., Amherst College, Amherst, Massachusetts.
POLLISTER, DR. A. W., Columbia University, New York City, New York.
POND, DR. SAMUEL E., Marine Biological Laboratory, Woods Hole, Massachusetts.
PRATT, DR. FREDERICK H., Boston University, School of Medicine, Boston, Massachusetts.
PROSSER, DR. C. LADD, University of Illinois, Urbana, Illinois.
RAND, DR. HERBERT W., Harvard University, Cambridge, Massachusetts.
RANKIN, DR. JOHN S., Zoology Department, University of Washington, Seattle, Wash.
REDFIELD, DR. ALFRED C., Harvard University, Cambridge, Massachusetts.
DERENYI, DR. GEORGE S., Department of Anatomy, University of Pennsylvania, Philadelphia, Pennsylvania.
REZNIKOFF, DR. PAUL, Cornell University Medical College, 1300 York Avenue, New York City, New York.
RICE, PROF. EDWARD L., Ohio Wesleyan University, Delaware, Ohio.
RICHARDS, PROF. A., University of Oklahoma, Norman, Oklahoma.
RICHARDS, DR. O. W., Research Department, Spencer Lens Company, 19 Doat Street, Buffalo, New York.
RIGGS, LAWRASON, JR., 120 Broadway, New York City, New York.
ROGERS, PROF. CHARLES G., Oberlin College, Oberlin, Ohio.
ROMER, DR. ALFRED S., Harvard University, Cambridge, Massachusetts.
ROOT, DR. R. W., Department of Biology, College of the City of New York, Convent Avenue and 139th Street, New York City, New York.
ROOT, DR. W. S., College of Physicians and Surgeons, Department of Physiology, 630 West 168th Street, New York City, New York.
RUEBUSH, DR. T. K., Osborn Zoological Laboratory, Yale University, New Haven, Connecticut.
RUGH, DR. ROBERTS, Department of Biology, Washington Square College, New York University, New York City, New York.
SASLOW, DR. GEORGE, Worcester State Hospital, Worcester, Massachusetts.
SAYLES, DR. LEONARD P., Department of Biology, College of the City of New York, 139th Street and Convent Avenue, New York City, New York.
SCHAEFFER, DR. ASA A., Biology Department, Temple University, Philadelphia, Pennsylvania.

SCHECHTER, DR. VICTOR, College of the City of New York, 139th Street and Convent Avenue, New York City, New York.

SCHMIDT, DR. L. H., Christ Hospital, Cincinnati, Ohio.

SCHOTTÉ, DR. OSCAR E., Department of Biology, Amherst College, Amherst, Massachusetts.

SCHRADER, DR. FRANZ, Department of Zoology, Columbia University, New York City, New York.

SCHRADER, DR. SALLY HUGHES, Department of Zoology, Columbia University, New York City, New York.

SCHRAMM, PROF. J. R., University of Pennsylvania, Philadelphia, Pennsylvania.

SCOTT, DR. ALLAN C., Union College, Schenectady, New York.

SCOTT, PROF. WILLIAM B., 7 Cleveland Lane, Princeton, New Jersey.

SEMPLE, MRS. R. BOWLING, 140 Columbia Heights, Brooklyn, New York.

SEVERINGHAUS, DR. AURA E., Department of Anatomy, College of Physicians and Surgeons, 630 West 168th Street, New York City, New York.

SHAPIRO, DR. HERBERT, Department of Physiology, Hahnemann Medical College, Philadelphia, Pa.

SHULL, PROF. A. FRANKLIN, University of Michigan, Ann Arbor, Michigan.

SHUMWAY, DR. WALDO, University of Illinois, Urbana, Illinois.

SICHEL, DR. FERDINAND J. M., University of Vermont, Burlington, Vermont.

SICHEL, MRS. F. J. M., State Normal School, Johnson, Vermont.

SINNOTT, DR. E. W., Osborn Botanical Laboratory, Yale University, New Haven, Connecticut.

SLIFER, DR. ELEANOR H., Department of Zoology, State University of Iowa, Iowa City, Iowa.

SMITH, DR. DIETRICH CONRAD, Department of Physiology, University of Maryland School of Medicine, Lombard and Greene Streets, Baltimore, Maryland.

SOLLMAN, DR. TORALD, Western Reserve University, Cleveland, Ohio.

SONNEBORN, DR. T. M., Department of Zoology, Indiana University, Bloomington, Indiana.

SPEIDEL, DR. CARL C., University of Virginia, University, Virginia.

SPENCER, DR. W. P., Department of Biology, College of Wooster, Wooster, Ohio.

STABLER, DR. ROBERT M., Department of Zoology, University of Pennsylvania, Philadelphia, Pennsylvania.

STARK, DR. MARY B., 1 E. 105th Street, New York City, New York.

STEINBACH, DR. HENRY BURR, Columbia University, New York City, New York.

STERN, DR. CURT, Department of Zoology, University of Rochester, Rochester, New York.

STERN, DR. KURT G., 333 Cedar Street, New Haven, Connecticut.

STEWART, DR. DOROTHY R., Skidmore College, Saratoga Springs, New York.

STOKEY, DR. ALMA G., Department of Botany, Mount Holyoke College, South Hadley, Massachusetts.

STRONG, PROF. O. S., College of Physicians and Surgeons, Columbia University, New York City, New York.

STUNKARD, DR. HORACE W., New York University, University Heights, New York.

STURTEVANT, DR. ALFRED H., California Institute of Technology, Pasadena, California.

SUMMERS, DR. FRANCIS MARION, Department of Biology, College of the City of New York, New York City, New York.

SWETT, DR. FRANCIS H., Duke University Medical School, Durham, North Carolina.

TAFT, DR. CHARLES H., JR., University of Texas Medical School, Galveston, Texas.

TASHIRO, DR. SHIRO, Medical College, University of Cincinnati, Cincinnati, Ohio.

TAYLOR, DR. WILLIAM R., University of Michigan, Ann Arbor, Michigan.

TEWINKEL, DR. L. E., Department of Zoology, Smith College, Northampton, Massachusetts.

TURNER, DR. ABBY H., Department of Physiology, Mount Holyoke College, South Hadley, Massachusetts.

TURNER, PROF. C. L., Northwestern University, Evanston, Illinois.

TYLER, DR. ALBERT, California Institute of Technology, Pasadena, California.

UHLENHUTH, DR. EDUARD, University of Maryland, School of Medicine, Baltimore, Maryland.

UNGER, DR. W. BYERS, Dartmouth College, Hanover, New Hampshire.

VISSCHER, DR. J. PAUL, Western Reserve University, Cleveland, Ohio.

WALD, DR. GEORGE, Biological Laboratories, Harvard University, Cambridge, Massachusetts.

WARD, PROF. HENRY B., 1201 W. Nevada, Urbana, Illinois.

WARREN, DR. HERBERT S., 1405 Greywall Lane, Overbrook Hills, Pennsylvania.

WATERMAN, DR. ALLYN J., Department of Biology, Williams College, Williamstown, Massachusetts.

WEISS, DR. PAUL A., Department of Zoology, The University of Chicago, Chicago, Illinois.

WENRICH, DR. D. H., University of Pennsylvania, Philadelphia, Pennsylvania.

WHEDON, DR. A. D., North Dakota Agricultural College, Fargo, North Dakota.

WHITAKER, DR. DOUGLAS M., P. O. Box 2514, Stanford University, California.

WHITE, DR. E. GRACE, Wilson College, Chambersburg, Pennsylvania.

WHITING, DR. PHINEAS W., Zoological Laboratory, University of Pennsylvania, Philadelphia, Pennsylvania.

WHITNEY, DR. DAVID D., University of Nebraska, Lincoln, Nebraska.

WICHTERMAN, DR. RALPH, Biology Department, Temple University, Philadelphia, Pennsylvania.

WIEMAN, PROF. H. L., University of Cincinnati, Cincinnati, Ohio.

WILLIER, DR. B. H., Department of Biology, Johns Hopkins University, Baltimore, Maryland.

WILSON, DR. J. W., Brown University, Providence, Rhode Island.

WITSCHI, PROF. EMIL, Department of Zoology, State University of Iowa, Iowa City, Iowa.

WOLF, DR. ERNST, Biological Laboratory, Harvard University, Cambridge, Massachusetts.

WOODRUFF, PROF. L. L., Yale University, New Haven, Connecticut.

WOODWARD, DR. ALVALYN E., Zoology Department, University of Michigan, Ann Arbor, Michigan.

YNTEMA, DR. C. L., Department of Anatomy, Cornell University Medical College, 1300 York Avenue, New York City, New York.

YOUNG, DR. B. P., Cornell University, Ithaca, New York.

YOUNG, DR. D. B., 7128 Hampden Lane, Bethesda, Maryland.

REGENERATION OF THE REPRODUCTIVE SYSTEM FOLLOWING BINARY FISSION IN THE SEA-CUCUMBER, HOLOTHURIA PARVULA (SELENKA) [1]

FRANK R. KILLE

(Dry Tortugas Laboratory, Carnegie Institution of Washington, and Edward Martin Biological Laboratory, Swarthmore College, Swarthmore, Pennsylvania)

Although it is a familiar fact that sea-cucumbers possess an extraordinary capacity for regeneration and often reproduce asexually, very little is known concerning the normal cellular basis of such reconstitution Of particular interest in this regard is the reported regeneration of gonads in adult sea-cucumbers and the question of the origin of germ cells. Investigators commonly state that following autotomy of the viscera or binary fission involving a loss of gonadal tissue, the sea-cucumbers rebuild the structures which they lack, yet we are indebted to Deichmann (1922) for the only specific data relative to the condition of the gonad. In *Holothuria parvula* (Selenka) and *Holothuria difficilis* (Semper) which have undergone transverse fission she reports briefly that "In specimens where all other organs were nearly as well developed as in undivided specimens, genital organs were absent, or very feeble, while an equally sized normal, undivided animal had a one centimeter long tuft." Since the entire original reproductive system remains in the anterior fission half, the poorly developed gonads within large specimens could be explained by any one or by a combination of the following processes: (1) an extreme periodic retrogression of the gonad which may or may not be related to asexual reproduction, (2) a delay in the normal development of the reproductive system of some individuals possibly related to an early onset of asexual reproduction, or (3) the late regeneration of a gonad within a posterior fission half which has reconstituted all other parts. None of these phenomena has been demonstrated for any echinoderm.

It is proposed, therefore, to investigate the extreme variation existing in the reproductive system of *Holothuria parvula* (Selenka) by means of dissection and microscopic examination. Data on the gonad

[1] With the support of Grant No. 396 from the Penrose Fund of the American Philosophical Society.

will be obtained from three different groups: (I) whole animals in which all other systems are apparently typical, (II) anterior fission halves which are regenerating new posterior ends, and (III) posterior fission halves which are regenerating new anterior ends.

Materials and Methods

Holothuria parvula (Selenka) is a convenient holothurian for the laboratory and for histological studies, since it rarely exceeds a length of about 6–7 cm. (For general anatomy and taxonomy consult Deichmann, 1930.) It is common over most of the West Indian region. Specimens were collected at Dry Tortugas in late June and July of 1937, and again in 1938 with the help of Dr. B. R. Coonfield. For comparative purposes, additional specimens were obtained in January from the Bermuda Biological Station through the courtesy of Dr. J. G. H. Wheeler.

In order to kill the animal with the viscera intact a small amount of Bouin's fluid was first injected into the coelomic cavity and the entire specimen was then immersed in the solution. Within a few minutes it was slit open, the coelomic cavity thoroughly washed out with fresh Bouin's, and the animal stored in Bouin's until dissected. As a rule, a good fixation of the reproductive system was obtained.

Three hundred and forty specimens representing both the summer and winter collections were used for the present study. They included 170 that were apparently whole, while 75 were obviously regenerating an anterior end, and 95 were regenerating a posterior end. All specimens were first dissected and the reproductive systems studied *in toto* with the aid of a dissecting scope ($\times 22$). Graded stages in the development of the gonad were then selected from specimens regardless of their size. Thirty-four of these were studied histologically by means of serial sections. A variety of stains was used including alum hematoxylin, Mallory's triple, iron hematoxylin, and alum cochineal. For a sharp nuclear stain, the iron hematoxylin was best. Counter stains of eosin and cotton blue [2] were particularly helpful in bringing out the cytoplasmic inclusions peculiar to the germ cells.

The Reproductive System of H. parvula

The gonad of *H. parvula* develops in and from the left side of the dorsal mesentery, just behind the calcareous ring (Figure 1). It may be divided into three major portions: (1) a single tuft of gonad tubules, (2) the gonad-basis from which these tubules originate and (3) nests

[2] I am indebted to my colleague, Ruth Jones, for this helpful suggestion.

of germ cells imbedded within a restricted region of the dorsal mesentery just anterior to the basis (Figure 1, GC).

In the normal young specimens of my collection, the gonad consists only of nests of germ cells. This is true for all specimens whose volume was less than 0.7 cc. and whose shape, diameter and development of tube feet clearly separated them from the regenerating halves of large specimens. All these normal young individuals had a maximum diameter of 6 mm. or less. In specimens whose volume was just above 0.7 cc. and whose maximum diameter was 7 and 8 mm., I found the first indication of tubule formation. The tubules always arise at the posterior level of the germ cell field in the form of small vesicles projecting into the coelom on the left side of the mesentery (similar to Figure 5). Apparently, they arise consecutively, for if more than one is present, they are graded in size, the posterior one being the largest. As these vesicles develop, they increase in size, and change their shape to that of a tubule which may or may not branch dichotomously. The origin of the tubules in *H. parvula* is therefore similar to the fundamental pattern described for other sea-cucumbers and the nests of germ cells are analogous to the strand-like "germinal cord" found in *Mesothuria intestinalis* by Théel (1901).

As the tubules increase in number, the basis from which they arise enlarges and likewise bulges into the coelom as a single large vesicle. By the time four tubules have originated from the gonad-basis, a gonoduct is present in the dorsal mesentery of all specimens of my collections. It takes its origin from the anterior face of the gonad-basis and extends obliquely forwards and upwards to open on the surface of the body wall. In fully grown specimens one can find the opening in a depression located in a mid-dorsal position about 7 mm. behind the base of the tentacles. Among five apparently whole (non-regenerating) specimens from Tortugas, each of which possessed only three immature tubules, the gonoduct extended to the body wall in three, was incomplete in one, and entirely absent in the other. It may be completely developed when only one gonad tubule has appeared. In the Tortugas collections seven such specimens possessed a gonoduct, while four did not. Thus considerable variation exists, but the gonoduct never develops until at least one gonad tubule has made its appearance.

The Extreme Variation of the Reproductive System in the Large Animals

While it might be expected that increase in the size and complexity of the maturing reproductive system would be correlated with an increase in size of the maturing specimens, it is surprising to find within the largest

specimens of the collection a graded series of stages typical of its development. The great variation in the reproductive systems of three large specimens is strikingly shown by comparing Figure 1 with Figures 2 and 4, and in two medium-sized specimens, by comparing Figure 6 with Figure 5. Each one of the specimens is much larger than the minimum size at which this sea-cucumber typically matures, yet the gonoduct may or may not be present and the gonad varies from a group of mature tubules to mere nests of germ cells imbedded in the dorsal mesentery. The poorly developed reproductive systems are like those in very young, immature specimens with a volume of less than 1 cc. With the exception of the reproductive system, there are no other striking variations in the anatomy of these large specimens. Without dissection they would unquestionably pass as fully matured individuals. Apparently the size of the gonad is not necessarily correlated with the size of the animal except during the typical development of the reproductive system in young specimens.

Variation in the Gonad Due to Periodic Resorption of Discharged Tubules

Several investigators have reported the fact that in certain sea-cucumbers the gonad tubules reach maturity, discharge their gametes, and then undergo regression. If this tubule resorption extended even periodically to the entire gonad, such retrogression could well explain the variation in the size of the gonads. Examination of the midsummer material revealed 11 clear-cut cases of resorption actually in progress.

Plate I [3]

Anterior ends of 5 preserved specimens (The Dry Tortugas, July 10). Left body wall has been removed to show the water vascular ring (W), anterior portion of the alimentary canal (A), with a portion of the dorsal mesentery including the gonad site.

Figure 1. Mature ovary of a very large specimen (7.0 cc.). O, oviduct; GC, nests of germ cells imbedded in the dorsal mesentery, and ovary consisting of 9 tubules originating from a common gonad-basis. × 8.

Figure 2. Extremely simple gonad in a very large specimen (7.2 cc.). Note absence of gonoduct. Nests of germ cells are present in the mesentery, but do not show in the photograph. × 8.

Figure 3. Gonad region of Figure 2, enlarged. × 20.

Figure 4. Nests of germ cells imbedded in the dorsal mesentery at the gonad site. No gonoduct nor tubules. Large specimen (5.3 cc.). × 8.

Figure 5. The origin of the first tubule at the extreme posterior end of a well-defined gonad primordium. Medium-sized specimen (3.3 cc.). × 8.

Figure 6. Mature testes. R, advanced resorption of the posterior tubule. Medium-sized specimen (3.5 cc.). × 8.

[3] The writer is indebted to Mr. John Spurbeck, The Johns Hopkins University Department of Biology, for aid in photography.

PLATE I

There were also nine cases among the specimens collected at Bermuda in January and there were many specimens in each collection in which certain posterior tubules had apparently been completely resorbed. Figure 6 shows a typical case of seven graded tubules followed by two others which are shorter than the seventh. The ninth tubule (R) was brown and finely wrinkled in contrast to the white smooth walls of the other tubules. Histological examination shows that this last tubule has been invaded by large numbers of phagocytic cells.

For our present purpose, it is important to stress that regardless of the number of tubules that are being resorbed within unregenerating specimens, the retrogression never extends to the three most anterior tubules, nor to the gonoduct. These anterior tubules are of course very small, but they never show any signs of being resorbed. Their small size is due wholly to their immaturity. Not a single stage in the resorption of a gonoduct was observed. Once formed, the gonoduct is evidently a permanent structure. The combination of a very few immature tubules plus a well-developed gonoduct apparently represents the extreme retrogression of the gonad in whole specimens (Group I). In the light of these data it is highly improbable that the extremely simple gonad which is occasionally found in specimens of maximum size can be due to a periodic gonad retrogression. That a periodic resorption of the oldest tubules produces considerable variation in the gonad is obvious, but we must look elsewhere for an understanding of the extreme variations found in animals of maximum size.

Variation in the Gonad and Asexual Reproduction

If asexual reproduction begins at an early stage in some individuals, it is possible that the normal development of the gonad might be delayed. It is pertinent to consider this possibility since Crozier (1917) working at Bermuda came to the conclusion that "if *H. captiva* (= *H. parvula*, Deichmann, 1930) undergoes division normally, it can occur only in very young stages." Deichmann (1922), on the other hand, found that 40 of the 82 specimens collected by Mortensen at Buccoo Bay, Tobago, B. W. I., were regenerating either a new anterior or a new posterior end. She reports no differential as to size. She also states (1930) that even some of the type specimens which are in the Museum of Göttingen give evidence of regeneration.

My findings are in accord with those of Deichmann. Out of 117 specimens collected at Bermuda (January), 51 were obviously regenerating either a new posterior or anterior end and another 15 were probably in very late stages of regeneration. Identifications in the field at

Tortugas (June–July) revealed that 10 to 15 per cent were regenerating one end of the body. This figure would have been much greater if a dissection had been made of each specimen, for the very early and the very late stages in regeneration are difficult to recognize externally. In the first instance, sufficient new tissue has not appeared, while in the second, the normal dark pigmentation and proportions of the body have been restored. Not only is this additional evidence that *H. parvula* normally makes use of binary fission in reproduction, but a study of the sizes of the regenerating Tortugas specimens indicates that fission normally occurs only in specimens ranging from about 30 to 60 mm. in length and from 5 to 14 mm. at their greatest diameter. Since none of the small specimens of my collections gave any indication of a recent fission, the evidence is against the retardation of normal gonad development by the early introduction of an asexual generation.

It is of interest to consider at this point whether the condition of the gonad bears any particular relation to the onset of fission among the large specimens. Dissection of specimens which are obviously regenerating new posterior ends (Group II) will provide the pertinent data. Since such individuals have fairly recently undergone transverse fission, a study of their gonads should reveal any correlation that exists. The examination of 50 Tortugas specimens regenerating posterior ends showed that development of the gonad was more advanced than in the collection as a whole, yet all stages of gonad development were represented. Since the data cited above show that fission occurs only among the larger specimens it is to be expected that in the ordinary course of events the gonads in Group II would be the best developed. Five of them possessed gonads consisting only of germ cell nests, while four had several immature tubules. In two specimens, resorption had progressed beyond that seen in any of the unregenerating specimens. In these, a large gonad-basis and a well-developed gonoduct remained but no tubules were present. Most of the remaining 39 specimens possessed seven to 12 well-developed tubules. Apparently no particular condition of the gonad seems to favor asexual reproduction. This holds for the Bermuda winter collection as well as the Tortugas summer collection. Evidently the few extremely immature gonads found in large-sized specimens of Group II must be related to the same factors which bring about this condition in apparently whole animals.

Evidence for the Regeneration of Gonads

Since the presence of extremely immature gonads in large-sized specimens cannot be accounted for on the basis of gonad resorption, nor of

gonad retardation, the condition must be due to the regeneration of a gonad within a posterior half resulting from the process of transverse fission. If this were true, it would be reasonable to suppose that specimens which are obviously regenerating new anterior ends would show the earliest stages in such regeneration. On the contrary, data obtained from the dissection of 75 specimens of Group III, and the microscopic examination of five specimens, give no indication of the re-establishment of any part of the reproductive system.

Direct evidence of gonad regeneration must therefore be sought among the specimens of Group I, which includes some animals that are actually fission halves in very late stages of regeneration. In these specimens, the complete sterility found in Group III should persist, or very early stages in gonad regeneration should be found. From the dissection alone, one might be led to think that sterility did exist. Out of 80 large (30 to 60 mm.) specimens from Tortugas, which were apparently not regenerating, there were 14 which possessed only an opaque spot or spots in the dorsal mesentery instead of a gonad (Figure 4). Histological analysis shows, however, that these opaque spots are nests of germ cells imbedded in the mesentery. They are in every respect identical with the germ cells found in this same region in the very young, immature sea-cucumber where we recognize them to be the first morphological indication of a reproductive system. Therefore, complete sterility does not exist except in specimens of Group III, all of which have undergone fission comparatively recently.

Furthermore, using only the medium to large-sized animals of Group I which in every other respect are apparently full grown, one can select a complete series of stages which will represent the typical development of the reproductive system: first, the germ cell nest mentioned above (Figure 4); then, the addition of one or two minute tubules springing from the gonad-basis (Figures 5 and 2); then, a gonoduct extending half way from the gonad-basis to the dorsal body wall; and finally, a complete gonoduct. If a great number of specimens were collected, one could confine himself entirely to the very largest specimens, yet obtain data on the development of the reproductive system as complete as that provided by selecting various sizes of the very small sea-cucumbers. This complete series of stages in the development of a reproductive system and the absence of sterility in large-sized animals, coupled with the indirect evidence mentioned above, establishes the fact that *H. parvula* can regenerate the reproductive system as completely as any other system.

It is obvious that experimental transections would provide direct evidence on regeneration. Such experiments would also determine

whether or not there is any preparatory step preceding normal fission involving germ cell migration. These experiments were tried, but unfortunately the lag in the regeneration of the gonad was too great to obtain positive results at Tortugas. My first attempts failed because experimental animals did not live longer than two weeks in our aquaria. It was discovered later that operated animals, returned to the sea floor in wire cages filled with coral, lived and immediately started to regenerate. Three weeks after the operations all systems in the new anterior end were being re-established with the exception of the reproductive system. Externally the newly regenerated portion extended as a small knob of tissue between 2 and 3 mm. in length, though the size of the regenerating halves varied from 13 to 25 mm. in length. New anterior ends regenerated at approximately the same rate as did new posterior ends. Judging from the scanty evidence provided by these specimens, at least several months must be required to re-establish completely normal proportions for the newly regenerated ends. Since this usually is accomplished before rudiments of a new reproductive system make an appearance, a much longer period must elapse before the gonad tubules are regenerated. From a study of a preserved collection, Deichmann (1922) was also of the opinion that the "genital organs seem to develop very late."

This tardy regeneration of the gonads is of particular value, however, in providing evidence that an alternation of generations does not necessarily occur. As reported above, among the 50 Tortugas specimens regenerating new posterior ends, five possessed poorly developed gonads consisting of germ cell nests only. This condition is evidence that two successive fissions have occurred. The second one has taken place before sufficient time has elapsed for the complete regeneration of the gonad. It is evident that under natural conditions the asexual mode of reproduction may be repeated without the intervention of the sexual process.

The Origin of the Germ Cells in Sea-Cucumbers

The germ cells of the gonad tubules normally arise from an aggregation of germ cells imbedded in a restricted region of the dorsal mesentery in all sea-cucumbers so far analyzed. *H. parvula,* however, can develop after fission new and fertile gonads from the dorsal mesentery at a point far posterior to the primary gonad. In order to determine whether the primary group of germ cells has made any direct contribution posteriorly to the site of the secondary gonad, a careful study was made of the dorsal mesentery within posterior halves just after fission had oc-

curred. If gonad tubules are entirely dependent upon the germ cells found in this restricted region of the dorsal mesentery, it might be expected that immediately before transverse fission occurred some natural preparatory step might occur. This could take the form of a backward extension of the primary aggregation of germ cell nests, in which case it would be visible under low powers of magnification. On the other hand, it might take the form much more difficult to trace, namely, the migration of individual germ cells. When the dissecting microscope revealed no evidence of such a contribution, serial sections were prepared in order to search for individual germ cells. In all five specimens so prepared, none was found. If the primary store of germ cells made any contribution to the posterior fission half, such cells must have lost every characteristic which had so sharply differentiated them from other cells of the mesentery at their original site.

When these negative results are coupled with certain histological data of a positive nature, the need for a revision in the traditional history of the germ cells in sea-cucumbers is apparent in so far as these regenerating specimens are concerned. Nothing is known concerning the early history of the germ cells in sea-cucumbers until late in larval life. At this time certain enlarged cells in a restricted region of the epithelium covering the dorsal mesentery burrow into the body of the mesentery to establish a fund of germ cells. The germ cells are then contributed to the gonad tubules as they arise and establish a germinal epithelium in each. No one has ever suggested that germ cells may also arise from cells in the mesenterial epithelium of the adult, yet my observations on regenerating *H. parvula* all point in this direction. It is not within the scope of the present paper to present cytological details related to the origin of germ cells from cells in the coelomic epithelium, but attention may be called to the main features: (1) In specimens which possess only germ cells at the gonad site, the coelomic epithelium in this region of the dorsal mesentery is hypertrophied on the left side, (2) single cells and sometimes groups of several cells, each possessing a large nucleus and much cytoplasm, may be seen projecting into the mesentery from the left coelomic epithelium, and (3) at more posterior levels of this restricted region of the mesentery, groups or nests of similar cells lie more deeply imbedded in the mesentery, asymmetrically related to the left coelomic epithelium but isolated from it. In each group, some cells can be identified unmistakably as germ cells. Even in later stages when the regenerating gonad consists of a group of very well-developed tubules, sections through the dorsal mesentery just anterior to the tubules will show proliferation from the left epithelium of the mesentery. I

cannot at present give the precise origin of the particular cells in the epithelium which invade the mesentery as germ cells.

Regeneration of the Gonad in Other Sea-Cucumbers

The experiments on *Thyone briareus* (Lesueur) (Kille, 1939) constitute the only other investigations on gonad regeneration in sea-cucumbers which have included any histological details. The capacity of this genus to regenerate gonads within a four-month period was tested by extirpation. When tubules only were removed nests of germ cells were retained, imbedded in the dorsal mesentery at the anterior end of the gonad-basis. New tubules arose at an abnormal rate to re-establish a complete gonad. Those instances of gonad regeneration briefly noted in the literature as occurring within a few months after loss of the main mass of tubules are probably cases of this kind. For example, in *Thyone briareus* and *Cucumaria grubi,* Torelle (1909) reported that "the reproductive organs are the last to regenerate in individuals in which these organs have been extruded." Bertolini (1932), working with *Holothuria tubulosa* D. Ch., stated that the gonad is probably regenerated, though it was impossible for her to tell whether the gonad that was expelled was the whole gonad or only part of it.

If the nests of germ cells as well as the tubules are extirpated, *Thyone briareus* does not regenerate a gonad within the period of four months. The long period required for gonad regeneration in *H. parvula* emphasizes the possibility that the failure of *T. briareus* to completely reconstitute a gonad under these conditions might be due to insufficient time. On the other hand, genera do vary in respect to capacity for regeneration. It would not be unusual to find that *H. parvula* which frequently reproduces by fission could regenerate the entire reproductive system including germ cells, but that *Thyone briareus,* which reproduces by sexual means only, could not.

Summary

1. The extreme variation in the reproductive system of large specimens of the sea-cucumber, *Holothuria parvula,* is investigated by means of dissection and microscopic examination.

2. As a result of frequent transverse fission, specimens are found in three conditions irrespective of the variation in the reproductive system: (I) apparently whole animals, (II) anterior fission halves which are obviously regenerating new posterior ends, and (III) posterior fission halves which are obviously regenerating new anterior ends.

3. Sterility existed in all animals of Group III, but in none of Group I.

4. In small specimens of Group I, the degree of development of the reproductive system is correlated with the size of the animal, but in specimens of medium size or larger all stages in the typical development of the system occur.

The simplest gonad consists of nests of germ cells imbedded in the dorsal mesentery which originate from cells in the hypertrophied left epithelium.

5. Extremely simple gonads and the absence of a gonoduct in large specimens of Groups I and II cannot be attributed to periodic resorption nor to a retarded development related to asexual reproduction, though resorption of the oldest tubules does account for considerable variation in the size of the gonad.

6. It is concluded that the simple gonads in large specimens represent early stages in the late regeneration of the reproductive system within posterior fission halves which have already reconstituted all other systems.

7. The condition of the gonad in Group II shows that no correlation exists between a particular condition of the gonad and the occurrence of fission, and that under natural conditions the asexual mode of reproduction may be repeated without the intervention of the sexual process.

LITERATURE CITED

BERTOLINI, F., 1932. Rigenerazione dell' apparato digerente nelle Holothuria. *Publ. Stazione Zool. Napoli*, **12**: 432–443.

CROZIER, W. J., 1917. Multiplication by fission in Holothurians. *Am. Nat.*, **51**: 560–566.

DEICHMANN, E., 1932. On some cases of multiplication by fission and of coalescence in Holothurians. *Vidensk. Medd. Dansk. Naturhist. Foren.*, **73**: 199–206.

DEICHMANN, E., 1930. The Holothurians of the western part of the Atlantic Ocean. *Bull. Mus. Comp. Zool.*, **71**: No. 3; 43–226.

KILLE, F., 1939. Regeneration of gonad tubules following extirpation in the sea-cucumber, Thyone briareus (Lesueur). *Biol. Bull.*, **76**: 70–79.

THÉEL, H., 1901. A singular case of hermaphrodism in Holothurids. *Bihang. till K. Svenska Vet. Akad. Handlinger*, Afd. IV, **27**: 1–38.

TORELLE, E., 1909. Regeneration in Holothuria. *Zool. Anzeig.*, **35**: 15–22.

PELAGIC LARVAL STAGES OF THE SAND CRABS EMERITA ANALOGA (STIMPSON), BLEPHARIPODA OCCIDENTALIS RANDALL, AND LEPIDOPA MYOPS STIMPSON

MARTIN W. JOHNSON AND WELDON M. LEWIS

(*Scripps Institution of Oceanography,*[1] *University of California, La Jolla*)

Introduction

In a previous report (Johnson, 1940) the larvae of *Emerita analoga* were dealt with from the standpoint of dispersal of pelagic stages as correlated with and as indicators of water currents off the California coast. That report was made possible only through a detailed study of the pelagic developmental stages which provided a key to the exact identification essential to investigation of the distribution.

The present report will cover mainly the descriptive details of the larval developmental stages of which there are five.

A study of the early larval stages of two other less abundant sand crabs, namely *Lepidopa myops* Stimpson and *Blepharipoda occidentalis* Randall, was undertaken by the senior author, and is here reported briefly for comparison. This study was made in order to ascertain whether or not the larvae of these related forms are sufficiently similar to those of Emerita to cause confusion when piecing together the developmental series by collecting the later zoeal stages from the plankton as was done for Emerita. That no such confusion is possible is seen from the strikingly different structure of the zoeal stages of each as shown in the figures.

Emerita analoga

Emerita analoga is abundant in the intertidal zone of many sandy beaches along the coast of southern California and according to Schmitt (1935) its range of distribution is from Oregon (Holms); Drake Bay, California, to Magdalina Bay, Lower California; and from Salvery, Peru, to Lota, Chile. A second Pacific species, *Emerita rathbunae* Schmitt, occurs from La Paz, Lower California, to Capon, Peru, but because of the remoteness from our region its pelagic larvae are not likely to be confused with larvae of *Emerita analoga* caught in our waters.

[1] Contributions from the Scripps Institution of Oceanography, New Series No. 170.

The larvae of two species of Emerita, namely *Emerita talpoida* of the eastern North American coast and *Emerita asiatica* of the Asiatic coast, have previously been investigated, both under the generic name *Hippa*. Of these, the former was studied incompletely by Smith (1877) and in greater detail by Faxon (1879) and the latter in detail by Menon (1933), who also makes comparisons between the two species.

As might be expected within the same genus, the structure of these larvae is very similar to that of our local species. The major differences will be noted in the course of discussion.

The eggs are carried attached to the abdominal appendages during the incubation period and the larvae, which quickly become pelagic, hatch in the first zoeal stage. A figure of this stage and also early cleavage stages of the eggs has been given by Johnson and Snook (1927).

Distribution of Larvae

In Table I is given an analysis of the occurrence of Emerita larvae for cruises covering the period April 8 to December 18. The seasonal distribution here suggested would indicate that the bulk of larvae are

TABLE I

Frequency of occurrence of Emerita larvae taken during the routine cruises of the "E. W. Scripps" for periods indicated

1938	Number of stations	Per cent successful for larvae	Zoea I	Zoea II	Zoea III	Zoea IV	Zoea V
April 8–12	7	0.0					
June 7–18	25	4.0			1		
August 16–26	34	38.0	9	30	12	12	1
October 26–November 5	28	36.0			6	25	
December 9–18	27	22.0			4	17	1

hatched during July and early August. This appears also to be in keeping with the conclusion of MacGinitie (1938) that the height of the mating season falls in May and June, though the duration of egg carriage by the female has not been determined. The wide distribution (Figure 1) indicates that the pelagic period is a relatively long one enabling the larvae to become dispersed and transported from their place of origin in the intertidal zone to distances of over a hundred miles seaward.

It is interesting that somewhat greater numbers of larvae were taken at stations relatively far from the coast, and the frequency of separate stations yielding one or more larvae is about the same here as in the

inshore area. These offshore stations fall in an extended area paralleling the coast as indicated by the line in Figure 1 enclosing locations where a total of six or more larvae have been taken. This is particularly striking since it is contrary to expectations in view of the coastal origin of the larvae and the diminution of numbers expected to accompany offshore dispersal. In seeking an explanation, it should be pointed out that it has been shown (Sverdrup and Fleming, 1941) that during

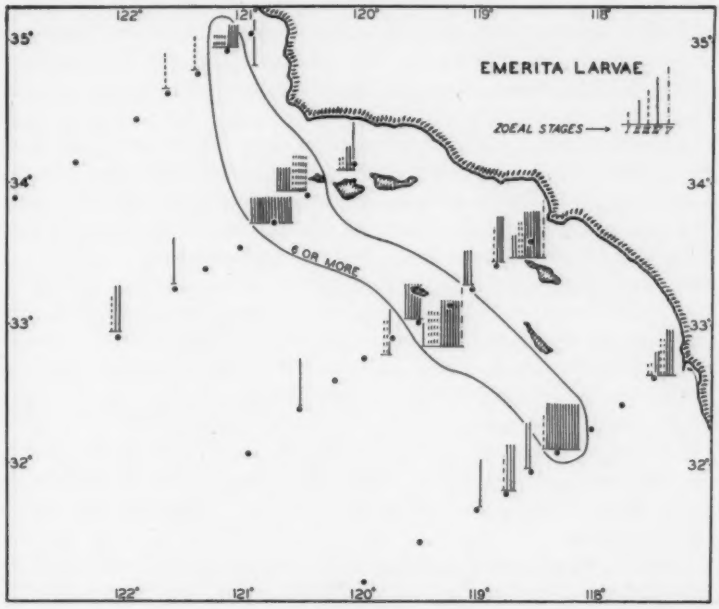


FIGURE 1

this period there exists a body of upwelled and mixed water streaking southward off-shore corresponding roughly to the area abundant in Emerita larvae. These waters are also notably productive in phytoplankton (Sverdrup and Allen, 1939) and in zoöplankton (Johnson, unpublished). Certain marine birds, e.g., the black-footed albatross, occur in greatest numbers in a long narrow band corresponding roughly to this area of increased organic production (Miller, 1940). It may therefore be suggested that, perhaps, the abundant plankton food supply makes this also a better region for survival of larvae than is true of the waters between this region and the immediate coast.

Studies of the ecology and offshore distribution of Emerita larvae in other areas are not available for comparison.

Methods

In the preliminary study, oviferous females were collected on the beach and kept in aquaria until the eggs were about to hatch, which is indicated by a change of color in the eggs from a bright coral red to a dull grayish-brown. At this stage an entire appendage with its egg mass was removed and placed in a glass culture chamber constructed from a 60 by 19 mm. shell vial. The bottoms of the vials were removed and replaced by a piece of number 8 × (average mesh aperture of 0.21 mm.) bolting cloth held in place by a rubber band. The tops were closed in a similar manner. Several culture chambers stocked with eggs were then placed end on end in a vertical glass tube approximately 45 centimeters long and 22 millimeters in diameter, or just sufficient to allow the shell vials to slip into place. The tube was held in a vertical position by a clamp and ringstand. Aerated sea water, pumped from the sea, was allowed to pass upward through the tube and culture chambers and overflow from the top. The rate of flow was regulated by screw clamps such that the eggs were almost held in suspension by its force. In this manner hatching usually occurred within a period of 24 hours and approximately half of the larvae hatched by this method were already free-swimming by this time. The young zoea are very helpless until their maxillipeds and their posterior setae are fully extended, for it is by means of these that the larvae are enabled to swim. Zoea which were hatched in the above manner lived without attention from six to eight days in the same culture chamber.

Feeding the larvae diatoms, ground Ulva or fresh plankton in the tubes failed to lengthen the period of life. Thus it was not possible to rear the larvae in the tubes sufficiently long to enable them to pass into the second zoeal stage; nevertheless, the method was the most satisfactory in caring for the eggs and bringing about a high percentage of hatching.

The best success in rearing the larvae beyond this age was realized in cultures consisting of a small number of individuals. Two free-swimming zoea were kept alive in a Syracuse watch glass for 34 days. The water was changed daily by means of a pipette and a portion of a fresh plankton haul, which had been strained through number 8 × bolting cloth to remove large and possibly harmful organisms, was fed daily. Similar success was also obtained with two zoea placed in a finger bowl and kept at approximately 18° C. in a constant temperature room which was totally dark. The water was kept in motion by a mechanical rocking

table, which was in constant operation throughout the period. One of the two larvae died on the twenty-fourth day without further change. The remaining one lived an additional ten days in which time it entered the second zoeal stage. Two long spines had developed from the posterior lateral margins of the carapace. These extended obliquely downward at an angle of about 45 degrees from the dorsoventral line and were approximately as long as the carapace was wide. The rostral spine had become greatly elongated. This specimen, unfortunately, soon died, but it showed the distinguishing features of the second zoeal stage and provided a key to following through the subsequent stages from specimens collected from the plankton as indicated below.

Some zoea of stages I and II were obtained from routine plankton hauls made with small nets at the Institution's pier and also from occasional samples collected at stations five to ten miles off Point Loma. Stages IV and V were, however, absent from all of these near-shore collections. The later stages and also most of stages II and III were collected off-shore, mainly in vertical plankton hauls taken from 200–0 meters with a 70 cm. net aboard the research vessel, "E. W. Scripps," at four lines of stations extending seaward to distances of 150 to 180 miles off the coast from San Luis Obispo to San Diego (Fig. 1).

Description of Zoeal Stages

stage i (Plate I, Figure 1)

Average sizes:

Maximum width of carapace	.53 mm.
Maximum length of carapace	.70 mm.
Length of abdomen including telson	.72 mm.
Length of rostral spine	.20 mm.

The average sizes given for this and all subsequent stages except Stage V are based on measurements of ten or more specimens. In Stage V only five specimens were available. The carapace measurements are used in preference to total length because the inclusion of the abdomen in the latter is not ordinarily practicable owing to its being strongly flexed in a position making exact measurement difficult.

Stage I is essentially similar to the first stage in the development of *E. talpoida* as given by Faxon and also that of *E. asiatica* as described by Menon. It is characterized by a short rostral spine and by the absence of lateral spines on the carapace. Slight thickenings mark the region where the latter will appear in the second zoeal stage. The eyes are conspicuously stalked and project laterally at right angles, slightly beyond the margin of the carapace. The carapace in this and subsequent stages is translucent, colorless and of uniformly smooth texture.

First Antennae (Plate II, Figure 1).—These are thick, short, unjointed processes bearing three aesthetes at the distal ends. No rudiments of the secondary flagellum were observed until the post-zoeal stage as was found also by Smith and Menon in *E. talpoida* and *E. asiatica* respectively.

Second Antennae (Plate II, Figure 6).—The antennae are each terminated by an outer spine-like process with a thickened base that is continuous with the main body of the antenna. There is also a smaller dentiform process and from near its base there issues a small setose spine which remains essentially unchanged throughout the following zoeal stages.

Mandibles (Plate II, Figure 11).—The mandibular blades bear from six to ten sharp teeth arranged in a row, the ventral tooth being always much heavier and longer than the others. These appendages remain practically unchanged except for general growth throughout the zoeal stages.

During metamorphosis to the adult state, the mandibular blades are lost. This is doubtless correlated with the radical change that occurs in feeding habit at this time, for in the adult state the animal has secondarily adopted the habit of remaining quiescent in the sand while the plumose antennae screen microscopic food from the outgoing waves.

First Maxillae (Plate II, Figure 13).—These are similar to the first maxilla in *E. asiatica,* but have one less seta on the endopod than in *E. talpoida.* The exopod is terminally cleft and each branch bears a stout setose spine. On the newly hatched zoea these spines are deeply imbedded. Branching off about half way down the outer margin of the exopod is a small lobe armed with a single long seta. The endopod bears a group of three partially imbedded setae. A single small seta is found proximal to this group and remains unchanged throughout the zoeal development. This was also found to be true of *E. asiatica* by Menon.

Second Maxillae (Plate II, Figure 16).—The second maxillae are similar in shape and arrangement to *E. asiatica* and *E. talpoida.* However, the number of setae on the scaphognathite differs from both of these species. Faxon shows a different arrangement of setae on the

PLATE I

Emerita analoga—zoeal stages I-V

FIGURE 1. Stage I (enlarged scale).
FIGURE 2. Stage I (same scale as following stages).
FIGURE 3. Stage II.
FIGURE 4. Stage III.
FIGURE 5. Stage IV.
FIGURE 6. Stage V.

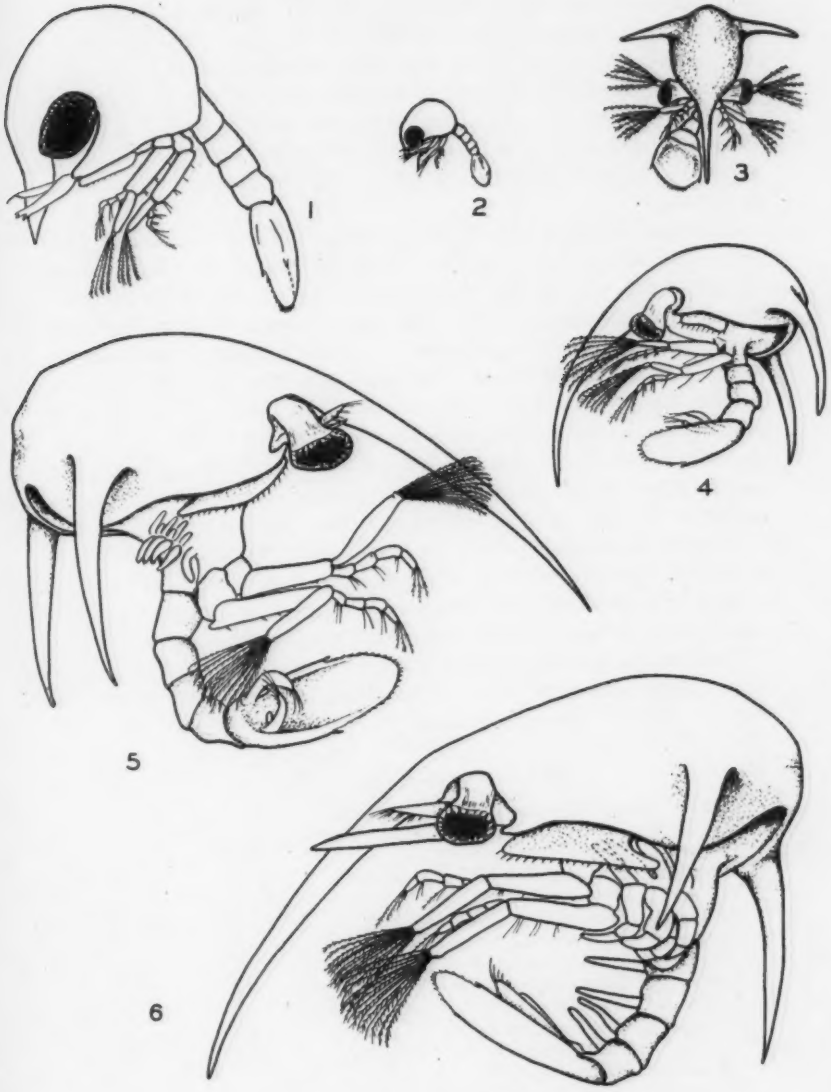


PLATE I

protopod for *E. talpoida*. The appendage of *E. analoga* is divided into two parts; the protopod bears a cluster of three short rudimentary setae accompanied by a fourth seta somewhat removed down the median margin. This isolated seta remains unchanged throughout the remainder of the developmental stages as is also the case in *E. asiatica*. The scaphognathite bears nine to ten setae along its anterior-outer margin. The posterior and inner margins are naked, differing in this respect from *E. talpoida*.

First Maxillipeds (Plate II, Figure 18).—These are essentially similar to those figured for *E. asiatica*. They are composed of a very short coxopod with a long basipod about equal in length to that of the endopod, including the three distal setae. The basipod bears six setae along the inner margin. The endopod consists of four segments each bearing setae. The first segment has three setae just below the joint, each of the next two has two in the same position. The distal segment has four setae of unequal length, the outermost two being the longest. No spinules as mentioned by Menon for *E. asiatica* were observed. The exopod consists of an elongated segment slightly longer than the endopod and a very short terminal segment bearing four long plumose setae.

Second Maxillipeds.—These are very similar to the first maxillipeds except that the distal segment of the endopod is relatively longer. The basipod is more slender and has less setae on its inner margin. The segmentation in both of these appendages remains the same throughout the later stages, the only point of difference, other than general growth, being in the number of setae on the distal segment of the exopod as

---

PLATE II

Emerita analoga—zoeal appendages

FIGURES 1 to 5. First antenna, Stages I to V.
FIGURES 6 to 10. Second antenna, Stages I to V.
FIGURE 11. Mandible, Stage I.
FIGURE 12. Mandible, Stage V (reduced scale).
FIGURE 13. First maxilla, Stage I.
FIGURE 14. First maxilla, Stage III.
FIGURE 15. First maxilla, Stage IV.
FIGURE 16. Second maxilla, Stage I.
FIGURE 17. Second maxilla, Stage V.
FIGURE 18. Maxilliped, Stage I.
FIGURE 19. Maxilliped, Stage V.
FIGURE 20. Telson, Stage I.
FIGURE 21. Uropod, Stage V.
FIGURE 22. Uropod, Stage IV.
FIGURE 23. Uropod, Stage III.
FIGURE 24. Arrangement of mouth parts, Stage V.

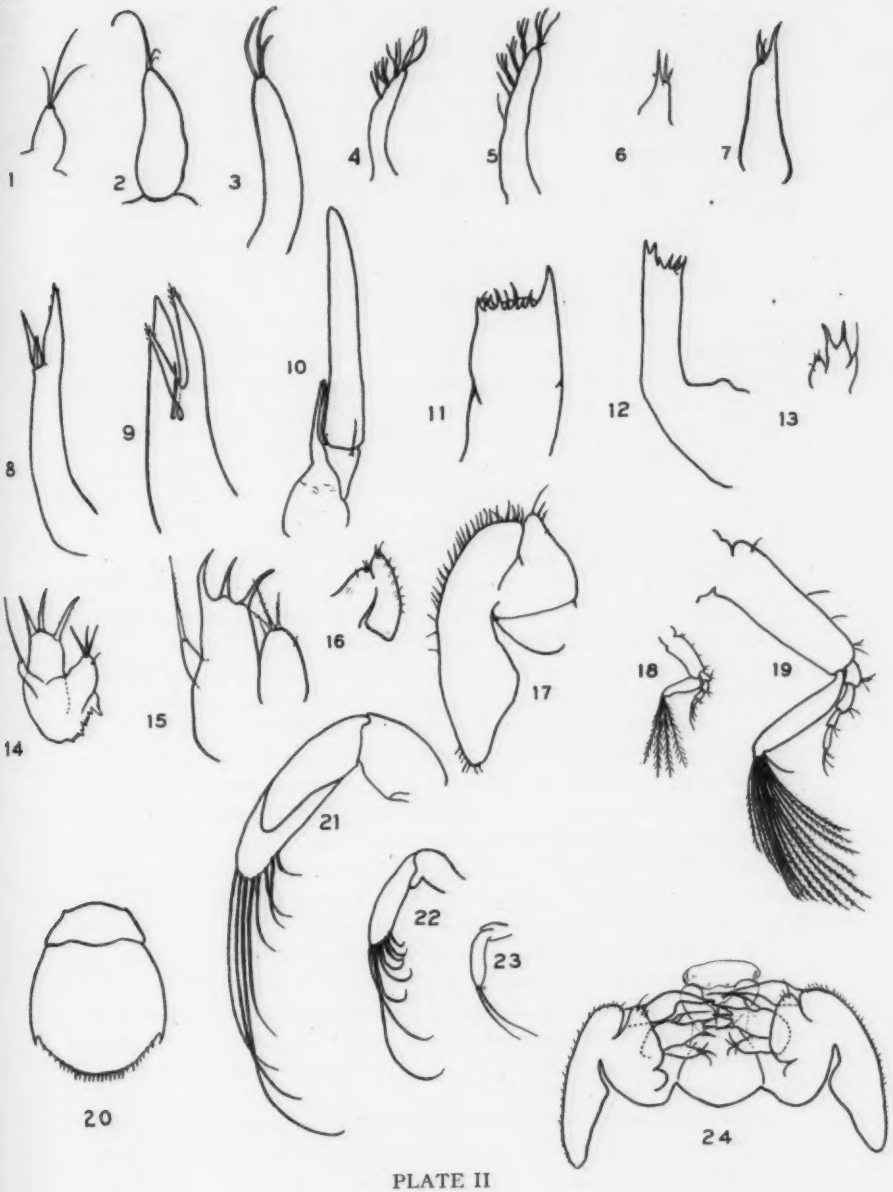


PLATE II

mentioned later. No rudiments of other thoracic appendages were found posterior to the second maxillipeds.

Abdomen.—The abdomen is composed of four free segments and the telson; the fifth and sixth segments are consolidated in the telson according to Smith. Proceeding from anterior to posterior the individual segments become shorter and markedly broader. No appendage rudiments appear either on the telson or on the abdominal segments until a later stage.

The telson (Plate II, Figure 20) agrees with *E. talpoida* but differs from the Asiatic species in that its width is slightly in excess of its length. However, there is a good deal of individual variation in this respect and in some it is fully as long as wide. The number of spines on the posterior margin is the same as that given for both *E. asiatica* and *E. talpoida.* This number, twenty-six, remains constant throughout the later stages.

STAGE II (Plate I, Figure 3)

Average sizes:

Maximum width of carapace	0.8 mm.
Maximum length of carapace	1.0 mm.
Length of rostral spine	1.2 mm.
Length of lateral spine	1.0 mm.

Stage II is characterized by the presence of two long, stout, posteriolateral spines nearly as long as the carapace is wide. These spines together with the rostral spine, which has increased to over three times the length attained in the preceding stage, form a tripod upon which the zoea is supported when resting on its ventral side. No additional appendages make their appearance in this stage. The carapace is no longer circular when viewed dorsally, but is now considerably longer than wide, and the eye stalks are projected more forward than in the previous stage.

First Antennae (Plate II, Figure 2).—Instead of three setae of about the same size, as found in Stage I, there are now two very much reduced slender setae and one long stout aesthete. *E. analoga* differs in this respect from both *E. asiatica* and *E. talpoida.*

Second Antennae (Plate II, Figure 7).—As in Stage I.

Mandibles.—As in Stage I.

First Maxillae.—The first maxillae now have six plumose setae issuing from the tips of the exopods.

Second Maxillae.—One additional seta is present on the margin of the scaphognathite.

First and Second Maxillipeds.—Each of these appendages now has six plumose setae on the tips of the exopods. No rudimentary appendages were present posterior to the maxillipeds.

Telson.—The telson is longer than wide. The number of spines on the proximal margin remains as in Stage I, but the number of denticles between the spines has increased.

STAGE III (Plate I, Figure 4)

Average sizes:

Maximum width of carapace	1.3 mm.
Maximum length of carapace	1.6 mm.
Length of rostral spine	2.1 mm.
Length of lateral spine	1.4 mm.

Stage III differs from the preceding stage mainly in size and in the presence of uropods on the telson. Two more setae make their appearance on the distal ends of the exopods of the first and second maxillipeds. The eyestalks have enlarged and now extend at an oblique angle, downward and forward. This is a continuation of the shift from a lateral position in the early stages to a position parallel to the median line as in the adult. In agreement with Menon, it appears that Smith's description and figures of what he (Smith) calls Stage two in the development of *E. talpoida,* correspond more closely to Stage III.

First Antennae (Plate II, Figure 3).—The first antennae have grown slightly longer and more slender, and bear three terminal aesthetes of unequal length.

Second Antennae (Plate II, Figure 8).—These are now slightly longer than the first antennae.

Mandibles.—As in preceding stage.

First Maxillae (Plate II, Figure 14).—As in preceding stage.

Second maxillae have eleven setae on the outer margin of the scaphognathite, being an increase of one over the previous stage.

First and second maxillipeds now bear eight plumose setae on the tips of the exopods, but there is no other change except general growth.

No other thoracic appendages appear posterior to the maxillipeds. Both Smith and Menon found rudiments of such appendages in *E. talpoida* and *E. asiatica* respectively. These rudiments may occur just prior to the molt to the fourth stage.

The *abdomen* is still without pleopods.

The *telson* is unchanged except for development of uniramous rudiments of uropods on its anterior ventral side (Plate II, Figure 23). These are each composed of a short unjointed basal segment and a long, curved, flattened lobe extending from it. This lobe becomes the exopod of the appendage in the later stages and is tipped with two long and one short setae.

STAGE IV (Plate I, Figure 5)

Average sizes:

Maximum width of carapace	2.0 mm.
Maximum length of carapace	2.4 mm.
Length of rostral spine	3.9 mm.
Length of lateral spine	2.2 mm.

This stage shows the first evidence of additional thoracic appendages and rudimentary pleopods on the abdomen. The number of plumose setae on the exopods of the first and second maxillipeds has increased to 16.

First Antennae (Plate II, Figure 4).—In addition to the three terminal aesthetes found in stage three, there are now eight more, distributed in two groups of three each and one group of two located on the inner side. Smith found only five or six in *E. talpoida* while Menon shows five for *E. asiatica.*

Second Antennae (Plate II, Figure 9).—These have grown considerably and the flagellum is now nearly as long as the dentiform processes. A small setose spine is situated at the base of the inner dentiform process. This appendage agrees generally with its equivalent in *E. talpoida* and *E. asiatica,* the difference being in the growth of the flagellum and in the number of spines on the dentiform processes.

Mandibles as in preceding stage.

First Maxillae (Plate II, Figure 15).—The inner lobe has elongated somewhat and bears one more small spine slightly proximal to the group of three on the distal end.

Second maxillae have now each 29 setae on the scaphognathite; an increase of 18 over the number in Stage III.

First and second maxillipeds differ from Stage III only in that the number of long plumose setae on the tips of the exopods have increased to 16. *E. asiatica* and *E. talpoida* each possess a total of only ten setae on these parts, which corresponds to lower Stage IV (see below) in *E. analoga.*

The additional thoracic appendages that are more fully developed in later stages appear as rudiments just posterior to the second maxillipeds. These consist of a third pair of maxillipeds and four pairs of legs which develop into walking legs in the first post-zoeal stage. Rudimentary gills appear above each pair of legs and are partially hidden by the margin of the carapace.

The *abdomen* is unchanged except for the presence of slight projections on the first four segments which were taken to be rudiments of the pleopods.

Uropods (Plate II, Figure 22).—The uropods have developed considerably over the preceding stage. The setae on the ends of each exopod have increased to eight which is three more than found in *E. asiatica* and four more than were reported in *E. talpoida*. These setae are of unequal length as was also reported for the other two species. Rudimentary endopods have appeared.

The *telson,* now narrower and longer, has no increase in number of marginal spines but the number of denticles between these has increased slightly. Three to six denticles were found between the spines near the median line and 25 between the last spine on the ventral margin and the large spine at the angle.

It should be mentioned here that a number of specimens were examined that appear to be intermediate between Stage III and Stage IV, being somewhat nearer the latter and may therefore, for convenience, be called "Lower Stage IV." It is not clear whether they represent a distinct instar between the above stages or are simply variables in Stage IV. In size they are comparable to the smaller specimens of that stage. The distinguishing features of Lower Stage IV are: ten setae on the tips of the exopods of the maxillipeds, a small precursory rudiment of the second antennal flagellum of later stages, absence of rudimentary thoracic appendages back of the second maxillipeds and no rudimentary endopod buds on the uropods.

STAGE V (Plate I, Figure 6)

Average sizes:

Maximum width of carapace	2.6 mm.
Maximum length of carapace	3.5 mm.
Length of rostral spine	4.2 mm.
Length of lateral spine	2.1 mm.
Length of abdomen, including telson	3.6 mm.
Width of telson	1.9 mm.
Length of telson	2.8 mm.

Stage V, which is the last zoeal stage, shows its greatest advancement over preceding stages mainly in the development of pleopods on all four segments of the abdomen and in the increased development of the thoracic legs. The flagellum of the second antenna is now conspicuous from a dorsal view of the animal.

First Antennae (Plate II, Figure 5).—The aesthetes have increased to fifteen, quite clearly arranged in groups: a terminal group of three long and one short, followed proximally by three successive groups consisting of three long aesthetes in each group. These in turn are followed by two solitary aesthetes. This arrangement is similar to that

found in the descriptions of Smith and of Menon for their species, but the numbers are greater by five in *E. analoga*.

Second Antennae (Plate II, Figure 10).—The flagellum has elongated to about three times the length of the dentiform processes. The distinct segmentation of the flagellum and of the first antenna as shown for *E. asiatica* was not in evidence but this condition becomes obvious only shortly before the zoea moults to the first post-zoeal stage.

Mandibles (Plate II, Figure 12).—No change.

First maxillae as in preceding stage.

Second maxillae (Plate II, Figure 17).—These now have 33 setae along the outer margin of the scaphognathite which agrees with the asiatic species. There are no setae on the inner margin.

First and second maxillipeds (Plate II, Figure 19).—These appendages show no increase in the numbers of setae on the exopods, the number remaining 16. The asiatic species has an increase from 10 in Stage IV to 12 in Stage V.

The other thoracic appendages are imperfectly segmented; the third pair of maxillipeds having three segments, and the legs each having a three-segmented basis and a one-segmented exopod, and a very rudimentary endopod.

The pleopods show no evidence of segmentation or other differentiations. They are still uniramous.

Uropods (Plate II, Figure 21).—The endopod is now approximately three-fourths the length of the exopod. The latter bears eight curved setae of unequal lengths. The endopod is unarmed. These appendages agree with the descriptions *E. talpoida* and *E. asiatica* except for the number of setae, the former having six present in this stage and the latter seven.

No attempt was made to rear the first post-zoeal stage from the fifth zoeal larva, and the numerous small specimens collected on the beaches were not studied sufficiently to warrant a detailed report. In view of the few fifth zoeal stages found in the plankton, and none of these being

---

PLATE III

Blepharipoda occidentalis—first zoea

FIGURE 1. Mandible.
FIGURE 2. First maxilla.
FIGURE 3. Second maxilla.
FIGURE 4. Larva, lateral.
FIGURE 5. Larva, dorsal.
FIGURE 6. Second antenna.
FIGURE 7. First antenna.
FIGURE 8. Second maxilliped.

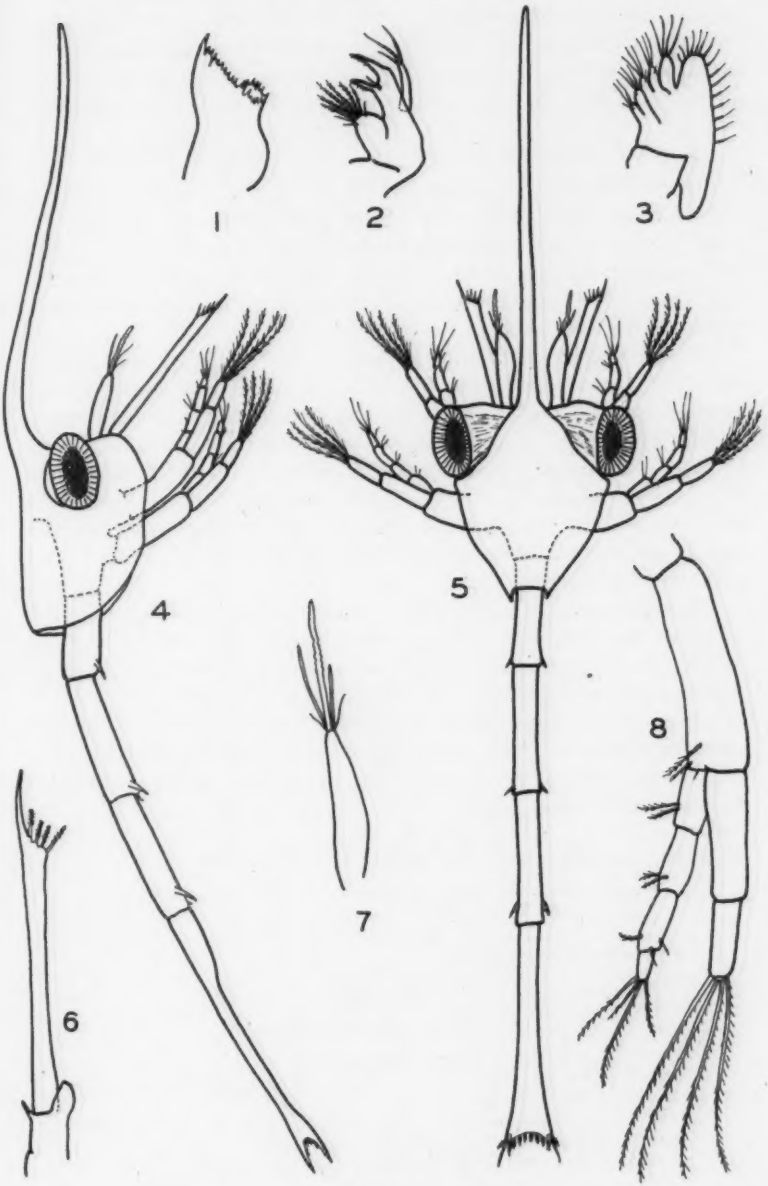


PLATE III

taken in the immediate vicinity of the beaches, it is a most profound mystery how these scattered planktonic stages or post-zoeal stages become sufficiently aggregated to account for the thousands of early post-zoeal stages constituting the large swarms found buried in the intertidal sands.

Blepharipoda occidentalis and Lepidopa myops

The adults of these two species occur in small numbers on sandy bottoms along the coast of southern and lower California. Though this study of their pelagic larval stages is incomplete, it is sufficient to enable distinguishing them from those of Emerita, and to make possible their identification for future studies on larval dispersal through water movements off the west coast.

The first zoeal larvae of Blepharipoda and Lepidopa were readily obtained by confining gravid females in small aquaria with sand and supplied with running sea water until the larvae hatched from the eggs which they carried. These stages are found sparingly also in the inshore plankton. Additional verification of the identity of the first stage of the former was also made from larvae reared by Mrs. S. Davis. No later larval stages have been obtained through rearing, but a plankton sample taken from 200 to 0 meters 30 miles off San Diego yielded a late zoeal stage of Blepharipoda.

Contrasted with the first zoea of Emerita, the corresponding stages of these sand crabs are strikingly distinctive. In Blepharipoda this is clearly reflected in the late stage as shown below, and the same is doubtless true also of Lepidopa. In Emerita it was necessary to know the anatomy of the second zoeal stage before the distinguishing features common to the later stages were recognizable. In that species the structure of the telson is the only striking characteristic carried forward to the successively older stages.

Blepharipoda occidentalis—first zoea (Plate III, Figures 1–8)

Carapace length not including spines	1.3 mm.
Carapace width	1.0 mm.
Rostrum	2.5 mm.
Abdomen including telson	3.5 mm.

Plate IV

Blepharipoda occidentalis—late zoea

Figure 1. Larva, dorsal (appendages omitted).
Figure 2. First antenna.
Figure 3. Second antenna.
Figure 4. Larva, lateral.
Figure 5. Urosome with uropod.
Figure 6. Third maxilliped.

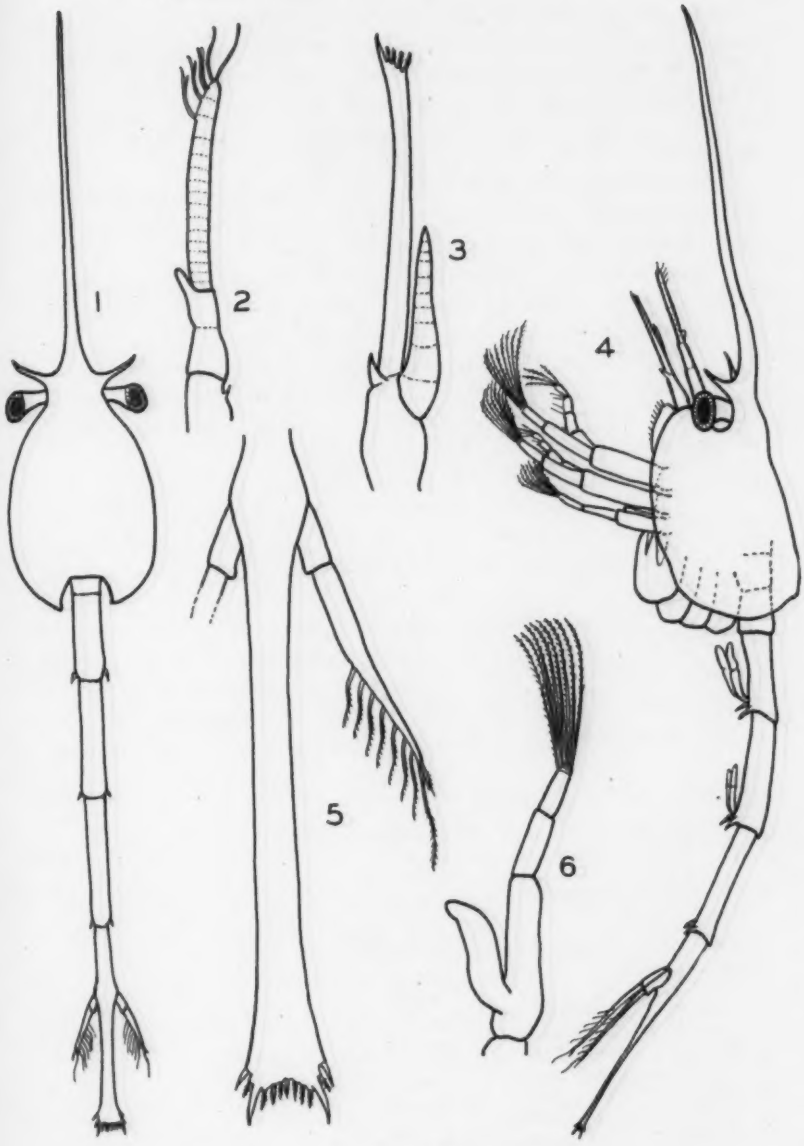


PLATE IV

The general features which sharply distinguish the larvae of this species from the corresponding stage in Emerita are the long rostral spine, the long, slender abdomen, and the narrow telson. It is separated from Lepidopa by the absence of lateral spines and by the character of the telson. Other details of the appendages as given below are also distinctive.

First Antennae (Plate III, Figure 7).—These appendages each bear a group of three terminal aesthetes and two small setae. They are relatively indistinctive in the three species discussed.

Second Antennae (Plate III, Figure 6).—These are very characteristic, each with a long distal segment, i.e. the scale or exopod, terminating in a curved spine-like process and four shorter setose spines on the inner side. The flagellum or endopod appears as a short rounded knob.

The *mandibles, first maxillae* and *second maxillae* (Plate III, Figures 1, 2 and 3) are in general not distinctive from the other species, though all are less rudimentary than in Emerita.

First and *second maxillipeds* are similar (Plate III, Figure 8). The exopod consists of two long segments and a very short terminal segment. Thus the segmentation differs from the other two species in that Emerita has but one distinguishable segment while Lepidopa has one long and two short segments. In each species however there are four long terminal setae. The endopod consists of four segments as in Emerita.

The *telson* (Plate III, Figure 5) is very narrow, with a thin concave posterior margin armed with two lateral smooth spines between which is a row of eight shorter setose spines. On the dorsal surface of the telson a short setose spine is situated at the base of each of the smooth spines.

Late zoea (Plate IV, Figures 1–6)

Carapace length not including rostrum	4.5 mm.
Carapace width	3.0 mm.
Rostrum	6.5 mm.
Abdomen including telson	10.0 mm.

PLATE V

Lepidopa myops—first zoea

FIGURE 1. Mandible.
FIGURE 2. First maxilla.
FIGURE 3. Second maxilla.
FIGURE 4. Larva, lateral.
FIGURE 5. Larva, dorsal.
FIGURE 6. First antenna.
FIGURE 7. Second antenna.
FIGURE 8. Second maxilliped.

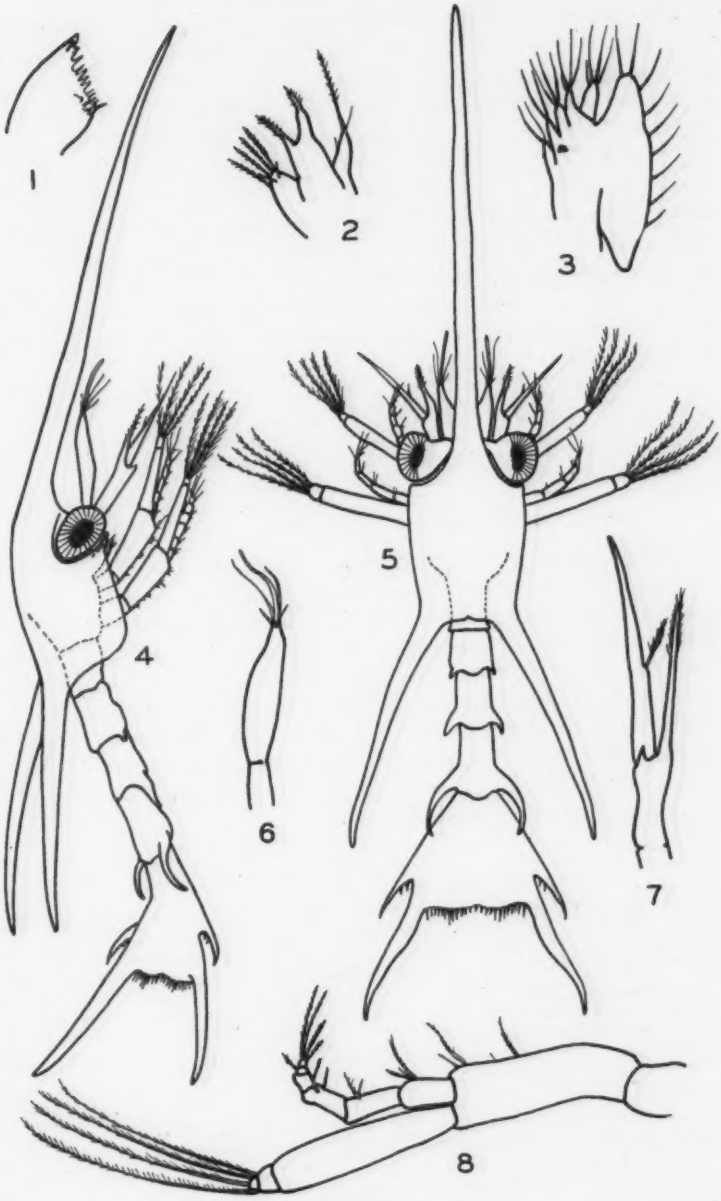


PLATE V

The most pronounced differences between this and the first zoeal stage are found in the development of (1), a pair of strong anteriorly directed spines on the carapace at the base of the rostrum; (2), a pair of slender uropods on the telson; and (3), third maxillipeds and rudiments of other thoracic and abdominal appendages as shown in Plate IV, Figure 4. The number of terminal setae on the exopod of the first, second, and third maxillipeds is eight. The scale or exopod of the second antenna has not changed but the flagellum has increased in length and shows segmentation under the skin. The telson armature has changed only by addition of a spine at each side of the posterior lateral angles. The eyes have become relatively much smaller and have very slender stalks, presaging the adult condition which perhaps will follow the next moult.

Lepidopa myops—first zoea (Plate V, Figures 1–8)

Carapace length not including rostral or lateral spines	1.2 mm.
Carapace width	0.8 mm.
Rostrum	2.8 mm.
Abdomen including telson	2.2 mm.

Distinguishing features characterizing this stage are: (1), the long rostrum; (2), carapace with long posterior lateral spines directed backwards and reaching well beyond the last abdominal segment; (3), relatively broad abdomen with conspicuous lateral spines which on the last abdominal segment are especially strongly developed; and (4), the very broad triangular telson provided with two pairs of heavy lateral projections between the larger of which is a series of 24 small spines. Smaller but distinctive features are found in the second antenna (Plate V, Figure 7) and the first and second maxillipeds (Plate V, Figure 8) the endopod of which consists of six distinct segments.

LITERATURE CITED

FAXON, WALTER, 1879. On some young stages in the development of Hippa, Porcellana and Pinnixa. *Bull. Mus. Comp. Zool. Harv.*, **5**: 253–268.

JOHNSON, MARTIN W., 1940. The correlation of water movements and dispersal of pelagic larval stages of certain littoral animals, especially the sand crab Emerita. Sears Foundation: *Jour. Marine Res.*, **2**: 236–245.

JOHNSON, M. E., AND H. J. SNOOK, 1927. Sea shore animals of the Pacific coast. Macmillan & Co., pp. 659.

MACGINITIE, G. E., 1938. Movements and mating habits of the sand crab, Emerita analoga. *Amer. Mid. Nat.*, **19**: 471–481.

MENON, M. KRISHNA, 1933. The life histories of decapod crustacea from Madras. *Bull. of the Madras Gov. Mus., New Ser.*, **3**: No. 3; 1–45.

MILLER, LOYE, 1940. Observation on the black-footed albatross. *The Condor*, **42**: No. 5; 229–238.

SCHMITT, WALDO L., 1935. Crustacea Macrura and Anomura of Porto Rico and the Virgin Islands. *Sci. Sur. Porto Rico and Virgin Is., New York Acad. Sci.*, **15**: part 2; 125–227.

SMITH, SIDNEY I., 1877. Early stages of Hippa talpoida, with a note on the structure of the mandibles and maxillae in Hippa and Remipes. *Trans. Conn. Acad. Sci.*, **3**: 311–343.

SVERDRUP, H. U., AND W. E. ALLEN, 1939. Distribution of diatoms in relation to the character of water masses and currents off Southern California in 1938. Sears Foundation: *Jour. Marine Res.*, **2**: 131–144.

SVERDRUP, H. U., AND R. F. FLEMING, 1941. The waters off the coast of Southern California, March to July 1937. *Bull. Scripps Inst. Oceanog.*, Univ. of Calif., **4**: 261–378.

PRECIPITIN REACTIONS AND SPECIES SPECIFICITY OF MONEZIA EXPANSA AND MONEZIA BENEDENI

MERLE M. MAHR

(*From the Department of Biology, University College, New York University*)

Introduction

Speciation has long been one of the difficult problems of biology. It is the purpose of this experiment to apply the precipitin test to the study of speciation. The value of the precipitin test in studies of this type lies in the fact that it is an objective test, reducing the subjective interpretations of the investigator to a minimum.

This study is a continuation of those done by Doctor R. W. Wilhelmi (1940). The author wishes to express his sincere appreciation to Doctor H. W. Stunkard under whose direction the work was done.

Material and Methods

The antigen was prepared by the same method as that of Wilhelmi (1940). The worms were washed through copious amounts of Ringer's solution. Following this treatment, they were washed through sterile physiological salt solution to reduce bacterial contamination, and finally through sterile distilled water to remove excess salts. The worms were frozen rapidly with dry ice, and then placed in a vacuum desiccator until dehydrated. The worm material was then removed from the desiccator and ground to a fine powder with an agate mortar and pestle. It was again placed in the vacuum desiccator to insure complete dehydration.

The lipids were removed from the powdered material by extraction for twenty-four hours with Bloor's solution at room temperatures. After the lipids had been removed, the saline-soluble material was extracted with a buffered physiological salt solution (pH 7.2 to 7.4). This was filtered through sterile fritted Jena glass filters. The clear, sterile filtrate contained the soluble proteins and carbohydrates of the worm material. The substances that did not go into solution, of course, constitute the saline-insoluble worm material. The concentration of soluble materials in the clear solution was determined by subtracting the weight of the insoluble residue from the weight of the lipid-free material.

The saline-soluble material was stored in the refrigerator until needed for injections.

For the production of antisera, injections were made into the lateral ear veins of healthy rabbits of the same age and weight. The antigen was injected in four doses, at three-day intervals. With each successive injection the dosage was increased, viz., 4 mg., 8 mg., 12 mg., and 16 mg., making a total of 40 mg. of antigen injected into each rabbit.

Two weeks after the last injection, blood was drawn by heart puncture, using a sterile syringe fitted with a large sterile hypodermic needle. The blood was allowed to clot and the serum, after centrifugation, was kept in the refrigerator in sterile flasks until needed for precipitin tests.

The precipitin reaction was performed using the ring technique. For each test 0.3 cc. of antiserum was carefully overlaid by 0.5 cc. of antigen. The dilution of antigen is expressed as grams per cubic centimeter.

Observations and Results

Homologous and Heterologous Titers*

Antiserum \ Antigen	*Monezia expansa*	*Monezia benedeni*
Monezia expansa	1 : 4000	1 : 1000
Monezia benedeni	1 : 1000	1 : 4000

* Tests were performed in triplicate. The antigens were prepared from several specimens of *Monezia expansa* and *Monezia benedeni*, respectively. In each case mature proglottids were removed from each of the strobila, fixed and stained in order to be certain of the specific identity.

The homologous titer of antisera developed against lipid-free, saline-soluble antigenic material for both *M. expansa* and *M. benedeni* was 1:4000. The heterologous titers for both species were 1:1000. The latter titers were confirmed by reciprocal tests.

Discussion

Wilhelmi (1940) reported: "'Species' of helminths may be defined tentatively as a group of organisms the lipid-free antigen of which, when diluted to 1:4000 or more, yields a positive precipitin test within one hour with a rabbit antiserum produced by injecting 40 mg. of dry-weight, lipid-free antigenic material and withdrawn ten to twelve days after the last of four intravenous injections administered every third day."

He tested nine species of helminths from nine different genera. The homologous reactions all took place at dilutions of 1:4000 or greater and none of the heterologous reactions would take place at a dilution as high as 1:4000. These experimental facts were the bases for his tenta-

tive definition of a species. However, he did not determine the heterologous titer of two species in the same genus.

In the present work the homologous and heterologous titers were determined for *M. expansa* and *M. benedeni,* two species in the same genus. The homologous titers for both species were 1:4000. The heterologous titers were 1:1000, confirmed by reciprocal tests. Therefore, the results of this experiment confirm the definition of a species advanced by Wilhelmi (1940) and demonstrate that the precipitin test is sensitive enough to differentiate between two species in the genus *Monezia.*

LITERATURE CITED

WILHELMI, R. W., 1940. Serological reactions and species specificity of some helminths. *Biol. Bull.,* **79**: 64–90.

PARAFOLLICULINA VIOLACEA (GIARD) AT WOODS HOLE

E. A. ANDREWS

(*Johns Hopkins University, Baltimore*)

The purpose of this communication is to show that the ciliated infusorian, *Parafolliculina violacea* (Giard), is a continuing member of the marine fauna at Woods Hole, Massachusetts.

The family, Folliculinidae, was formed to separate from the stentors these one-celled animals that have greater perfection of feeding apparatus through development of right and left lobes giving bilateral symmetry to a creature generally sedentary. In the life cycle there are frequent transformations from sedentary to motile phases and back again, and it is notable that the complex feeding apparatus of the sedentary phase undergoes complete involution before the motile phase is formed. The motile phase may be the whole body or only half of the body of the sedentary phase. The greatly simplified motile phase is like a larva, but, free swimming, it distributes the species and soon regrows the needed feeding apparatus before it functions as the next sedentary form.

While the sedentary form is highly contractile and irritable, it is the swimming form that shows special responses in selecting its site for building its follicle. In some species such responses lead to the sedentary form being found only in very restricted habitats, and in others lead to association of individuals in colonies.

The number of species in the Folliculinidae was judged to be 25 by Kahl in his review of the ciliated protozoa, in 1930–35. Most of these were described from the coasts of Norway and France, and from the Adriatic.

The Folliculinidae known at Woods Hole, Massachusetts, are all species previously described from Europe, and they have been spoken of under the following eight names: *Semifolliculina boecki* (Clap. Lach.) and *Parafolliculina violacea* (Giard) (Andrews, 1921); *Folliculinopsis producta, Parafolliculina amphora, Folliculina elegans,* and *Folliculina simplex* (Fauré-Fremiet, 1936); *Folliculina elegans, F. aculeata,* and *F. viridis* (Dewey, 1937). Moreover, in 1915, Dons stated that his *Semifolliculina spirorbis* of Norway was also found on materials from the east coast of North America and this may well have been Woods

Hole. But granting nine names so far accredited to Woods Hole, some are synonyms, and four or five species may be all known there.

One of these is quite different from the rest and unusually well characterized, *Parafolliculina violacea* (Giard), but as yet credited to Woods Hole merely on observation of one specimen, by the present writer in 1921. Renewed examination of that specimen occurring with several *Semifolliculina Boecki* Dons attached to Obelia mounted early in this century, along with study of four like ones mounted about the same period, has been supplemented with examination of two specimens on Obelia attached to laminaria from Woods Hole in late summer of 1941.

Students of this group agree that the very resistant, horny or chitinous follicles secreted by the swimming form furnish in their shapes and dimensions good specific and even generic characters.

The illustration (Figure I) represents two views of one follicle with enclosed sedentary animal as preserved with the Obelia in late summer of 1941, and so subjected to action of epsom salts followed by formalin with distortion of the form of the animal.

The full face and the profile views differ so much that older observers thought there were two forms of follicle but Fremiet saw that these were but the two aspects of any one follicle.

Standing erect, firmly attached by a rounded disk, the follicle resembles a bottle with body, or sac, much darker than the tube or neck which swells out right and left where it joins onto the body. This swelling has been called annular, but profile views show little of it. The body of the bottle is much flattened toward its top, and broad from side to side.

The cavity of the neck expands as the vestibule where it joins the body of the bottle and partakes of its flattening so that in two instances the vestibule was 50 micra wide but only 30 or 37 micra deep. In this region are the characteristic and unique valves formed by a membrane rising up from the edges of the body inside the vestibule, and with the same dark tint.

This membrane projecting into the vestibule cavity has a slit along its middle from right to left which is commonly held nearly closed by the elastic membrane but can be opened by the emerging animal and in this illustration one rather plasmolized arm of the animal is seen sticking up through the slit in the membrane. When the animal withdraws into the body of the follicle, the collapsing dorsal and ventral halves of the membrane act as valves to check intrusion from above but readily yield to pressure of the elongating animal that is to reach up its arms into the outside water to collect food.

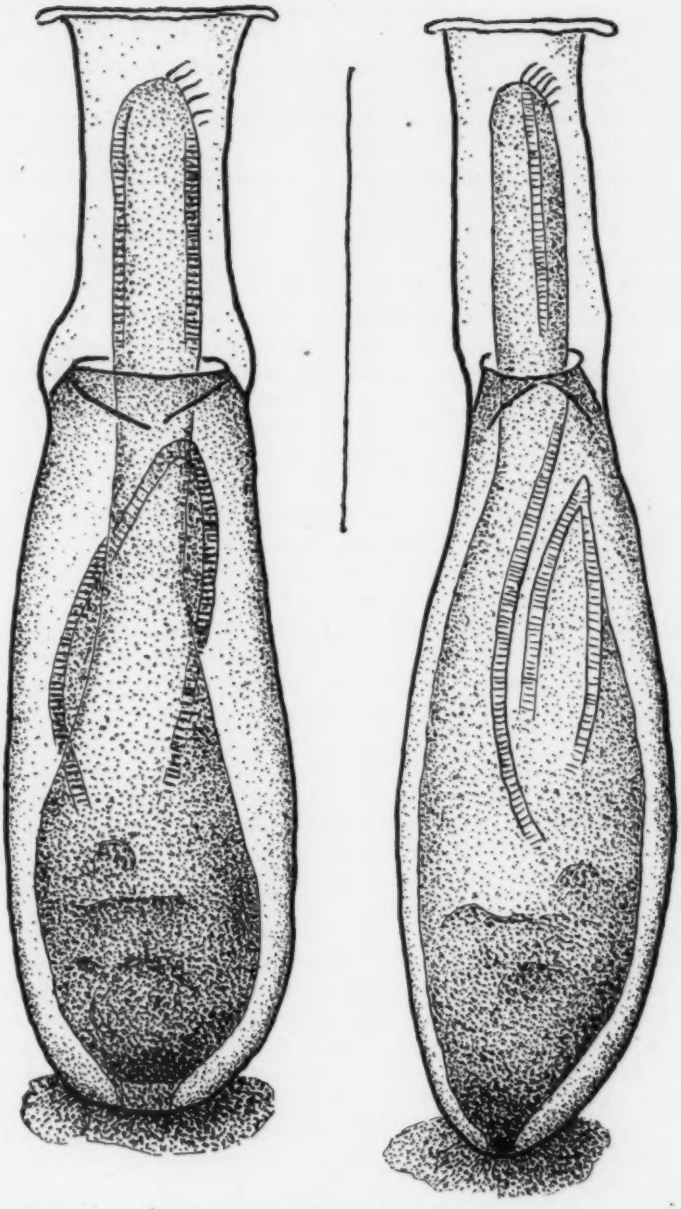


FIGURE I. Full-face and profile views of one follicle with preserved animal taken on Obelia and laminaria at Woods Hole, Massachusetts, in late summer of 1941. The line represents 100 micra.

Fremiet observed that the valve membrane was made as an afterthought by the swimmer that first secreted the body, then the neck, then the rounded collar or lip and, finally withdrawn down into the body, secondarily secreted an inner continuation of the body wall as the above membrane, which might be likened to an inner collar and was made by the collar-making organ of the swimmer.

Typically, the color of this species is not the usual green but a rare reddish-blue or violet. In these Woods Hole specimens, after preservation, the color contains a red element that makes the follicle brown or olive, while the attaching disk is darker and sometimes violet.

Both Hans Laachmann and Dons found a duplication of the upper half of the wall of the sac as an inner mantle that continues the valve membrane downward. Two of the Woods Hole specimens show this mantle in full face view but in profile only as creases. It was not observed in the specimen here illustrated, however, and may not be a constant character.

Another variable is the form of the lower end of the sac which in these few specimens is rounded, as in our illustration, and in most of the follicles figured by Dons, though Fremiet found flat bases in France and Hadzi shows one with a very flat base. He even found follicles reclining and fastened all along the side face of the body or sac.

The neck and vestibule of the follicle we illustrate was closely entwined by three turns of a clear spiral; although this suggested the spiral ridge known in some other species, we interpret it as accidental investment by a filamentous plant.

Of the nine specimens from Woods Hole, three were empty follicles and the others showed but remnants of the animal, yet some of these, as in the figure, show the chief anatomical features. Some of them revealed the usual spheroidal nucleus not seen in the one illustrated. Most all these were found associated with Obelia on laminaria from 5–10 feet depths and were in company with *Semifolliculina boecki* of Dons. Elsewhere this species occurs on various substrates but it may be noted that Dons found several dozens widely scattered on laminaria on the coasts of Norway, and Giard found them on laminaria on the coast of France. Failure to find this species on large collections of laminaria with Obelia and other hydroids and bryozoa collected at Woods Hole in January and in March 1942 adds to the suspicion that this species vanishes from shallow waters in winter.

The general uniformity in size and proportion of these animals is shown in the following measurements [1] of seven follicles, of which the first is that mentioned in 1921:

[1] All dimensions given are in micra.

Total length: 250, 250, 225, 230, 288, 240, 245.
Length of sac with valves: 168, 165, 150, 155, 168, 157, 152.
Width of mouth and collar: 42, 37, 32, 42, 45, 40.
Width of neck: 30, 32, 25, 42, 33, 35, 33.
Width of vestibule: 51, 42, 50, 52, 50, 50, 48.
Greatest width of sac: 63, 58, 62, 63, 60, 60, 65.
Greatest depth of sac: 50, 63, 58, 50, 63, 63, 50.
Least depth of sac: 25, 25, 32, 25, 33, 30, 25.

Also, the diameter of the nucleus was 18 and 25 in specimens third and fifth, and the diameter of the attachment disk was 35, 50, 37, 23, in the last four. The height of the valves was 12 and 13 in the fifth and the seventh.

While previous measurements made by authors do not include so many dimensions, it is evident that these Woods Hole specimens fall well in line with the others of this species. Thus Dons in Norway found that the total lengths ran from 260–310; Fremiet in France 230–260; Laachmann in Australia 200–260. The sac length given by Fremiet was 155–180. Its greatest width 75 in France and 60–70 in Australia, 55–90 in Norway. The only other measurements were greatest depth of sac in France 60; greatest width of mouth in Australia also 60. Laachmann states the nucleus measured 15.

Parafolliculina violacea (Giard) has been found on the north and the west coasts of France, on the Norwegian coast, in the Adriatic and on the west coast of Australia, as well as Sumatra. On this side of the Atlantic it occurs not only at Woods Hole but at Beaufort, North Carolina, in Chincoteague Bay, near the mouth of York River and in Tangier Sound of the Chesapeake Bay, and on the north coast of Long Island; as will be shown in other papers.

Summary

Parafolliculina violacea (Giard) was living associated with other Folliculinids and Obelia at Woods Hole, Massachusetts, early in this century in the summer season in different years. It was again found in small numbers on Obelia and laminaria in late summer of 1941.

In all essential characters and measurements these specimens agree well with those previously known from Europe. Failure to find any on material collected at Woods Hole in January and March 1942 may mean that this species, like some others of this family, has a winter season of scarcity.

LITERATURE CITED

ANDREWS, E. A., 1921. American Folliculinas: taxonomic notes. *Amer. Nat.*, **55**: 347–367.

CLAPAREDE AND LACHMAN, 1858. Études sur les Infusoires et les Rhizopods. I. Geneve.

DEWEY, VIRGINIA C., 1937. Test secretion in two species of Folliculina. *Biol. Bull.*, **77**: 448–455.

DONS, CARL, 1915. Karaffeldyrene. Naturen's Maihefte.

FAURÉ-FREMIET, E., 1936. La famille des Folliculinidae (Infusoria heterotrichida). *Mem. Mus. Roy. Hist. Nat. de Belgique*, **2, 3**: 1130–1173.

GIARD, A., 1888. Fragments biologiques XIII. *Bull. Scient. de la France et de la Belgique*, 3. Ser., **1**.

HADZI, JOVAN, 1935. Ueber die Xenökie der adriatischen Folliculiniden. *C. R. du XII-e Congres. Inter. d. Zool.* Lisbourne.

HADZI, JOVAN, 1938. Beiträge zur Kenntnis der adriatischen Folliculiniden (Inf. heterotricha). Subfamillie Eufolliculininae. *Acta Adriatica*, **ii**: 1–46.

KAHL, A., 1930–35. Tierwelt Deutschlands. Urtiere oder Protozoa.

LAACHMANN, H., 1901–03. Zur Kenntnis der heterotrichen Infusorien der Gattung Folliculina Lam. *Deutsche Südpolar-Expedition*, **XII**: Zool. IV.

A NEW SPECIES OF ECTOCHAETE (HUBER) WILLE, FROM WOODS HOLE, MASSACHUSETTS

FRANCESCA THIVY

(University of Michigan, Ann Arbor)

Nomenclature of the Genus

In 1892 Huber described two new species of algae under the names *Endoderma leptochaete* and *Endoderma Jadinianum* (b, pp. 319–25). In recognition of the fact that these species have setae, he placed them in the section Ectochaete, along with *Endoderma endophytum* (Möbius) Huber, (*Bolbocoleon endophytum* Möbius, 1891, p. 192). Wille (1909, p. 79) raised the section Ectochaete Huber to the position of a genus. He held the view that *E. Jadinianum* Huber was synonymous with *E. endophytum* (Möbius) Huber. A complete description of the genus may be framed as follows:

Ectochaete (Huber) Wille. (Bolbocoleon Möbius *pro parte,* Endoderma Lagerheim *pro parte*). Thallus microscopic, disklike or consisting of interwoven filaments, endophytic in the cell wall of larger algae or imbedded in the slime between the loose cortical filaments of brown or red algae; branching monopodial, but sometimes pseudodichotomous; branches free peripherally and more or less pseudoparenchymatous in the center of either the disk or mass of interwoven filaments; cells uninucleate, cylindrical to round, diameter 5–30 μ, length 1–5 times; many, or at least some, of the cells each provided with a long straight seta; seta not separated by a cross wall from the subtending cell; chloroplast parietal, platelike forming an incomplete girdle, and showing a few perforations occasionally; pyrenoids 1 to about 8; reproduction taking place by biflagellate zoospores or gametes; zoosporangium or gametangium usually saclike, lacking a seta but having a wide neck of varying length; gametes syngamous or parthenogenetic; zoospore, gamete, and zygote attaching themselves to the substratum and germinating directly; in the species growing within the cell wall, germination taking place from the anterior end of the zoöid along the long axis, but occurring at the posterior end at an angle to the long axis of the zoöid in the species found between the free cortical filaments of the host.

A KEY TO THE SPECIES OF ECTOCHAETE (HUBER) WILLE

1. Marine 2
1. Fresh water 3
2. Endophytic inside cell wall of certain green, brown, and red algae; setae few, delicate, with a constriction at the base; cells 5–15 μ diam. by 1–3 times; pyrenoids 1–3 .....*E. leptochaete*
2. Endophytic in slime between cortical filaments of certain brown and red algae; setae numerous, firm, without a constriction at the base; cells 8–18 μ diam. by 1–5 times; pyrenoids 1–8 .....*E. Taylori*
3. Endophytic inside cell wall of certain green algae; setae usually few, lacking a constriction at the base; cells 8–30 μ diam. by 1–5 times; pyrenoids 1–6 .....*E. endophytum*

Localities

E. leptochaete (Huber) Wille. Croisic, Bretagne, Huber in Sept. on a species of *Chaetomorpha*. L'Étang de Thau, Huber in Apr. on Cladophora spp., Chaetomorpha and *Ceramium diaphanum* (Lightf.) Roth. Banyuls, ile Grosse, cap du Troc, Feldman, May to June on *Dictyota dichotoma* and *Dilophus fasciola*. Tatihou, Hariot on *Cladophora tenerrima*. Devon, England, Newton, on *Ectocarpus penicilliformis, Ceramium diaphanum* and *Cladophora*.

Geographical distribution: W. Meriterranean: Atlantic coasts of England and France.

E. endophytum (Möbius) Wille. Heidelberg Bot. Gardens, Germany, Möbius on *Cladophora fracta* Kütz. growing in fish ponds. Albères, Pyr.-Or., Jadin and Huber in April and May on *Cladophora*.

Geographical distribution: Europe.

E. Taylori n. sp. Woods Hole, Mass., July to Sept. on *Mesogloia divaricata* (Ag.) Kütz., *Leathesia difformis* (L.) Aresch. and *Nemalion multifidum* (Weber and Mohr) J. Ag.

Ectochaete Taylori sp. nov. Punctiformis, 0.3–0.65 mm. diametiens vel annuliformis et minus quam 7 mm. lata, endophytica, inter filamenta algarum hospitalum penetrans; filamentis ramosis saepe inter se conjunctis pseudoparenchyma formantibus; cellulis cylindricis vel sphaericis, 8–18 micra crassis, 1–5 longioribus quam crassis, uninucleatis; chromatophoro singulo, planato, parietali, pyrenoideis 1–8 praedito atque seto longissimo nec basi septato non constricto; gametangio sacciformi vel irregulari, 8–18 micra crasso, longitudine usque ad quintuplo longiore quam crassiore, ostiolo tubuliformi lato praedito et numerosos zoosporos sexuales biflagellatos emittente, eosdem germinatione epiphyticos.

Habitat in algis variis Phaeophyceis et Rhodophyceis, videlicet Mesogloia divaricata, Leathesia difformi et Nemalio multifido.

Habit endophytic; thallus punctiform, 0.3–0.65 mm. in diameter, or annulate, 7 mm. or less in width, filamentous, embedded in the jelly

present between the cortical filaments of the host; filaments laterally branched, interwoven, partly fused; cells isodiametric to cylindrical, 8–18 microns in diameter, usually 1–5 times as long, uninucleate, pyrenoids 1–8; chloroplast parietal, forming an incomplete ring, platelike; most of the cells bearing a dorsal seta; setae conspicuous, long, straight, not constricted at the base and not separated by a septum from the subtending cell; usually 2.66 microns in diameter, up to 0.8 mm. in length; gametangium saclike or variously shaped, agreeing in diameter with vegetative cells, 1–3 diameters in length, provided with a wide neck about 8–18 microns in diameter and ⅓–5 times as long, with numerous biflagellate, syngamous or parthenogenetic gametes; germination epiphytic in type.

The author has taken the privilege of naming the new species described above after Dr. Wm. R. Taylor, who is closely associated with the phycology of the region of Woods Hole.

Occurrence

The endophyte was restricted to calm bays and grew near low-water mark, appearing when the hosts are mature and taking about four weeks to reach its fruiting stage. The germlings were seen in the last week of July, in 1939 and 1940, in *Mesogloia divaricata* (C. Ag.) Kütz., and fruiting commenced in the last week of August; in September there was evidence that the new crop fruited in a shorter time, namely from two to three weeks.

In *Nemalion multifidum* (Weber and Mohr) J. Ag., fruiting and germinating stages of the endophyte were seen together in 1940, from the middle of August up to the disappearance of the host in the beginning of September. In *Leathesia difformis* (L.) Aresch., the endophyte was in fruiting condition at the beginning of August, 1940 and 1941, and it was still present when the host disintegrated in the middle of the month.

In the summer of 1941 Mesogloia and Nemalion did not appear on Rocky Beach. Early in the summer the rocks were found covered by *Mytilus edulis* L., which perhaps had a harmful effect on the microscopic phases of these hosts.

Winter Phase of the Endophyte

The above hosts are summer annuals that pass through the winter in the form of microscopic plantlets. In *Leathesia difformis* (L.) Aresch. the zoöids of the plurilocular sporangia were found by Sauvageau (1925, pp. 1633–35) to grow into microscopic thalli, and their recapitulation to the third generation by means of apparently asexual plurilocular spo-

rangia was noticed. Kylin (1933, pp. 64–68) obtained microscopic phases from both kinds of zoöids. The plantlets in his cultures coming from unilocular sporangia were sterile, while those from plurilocular sporangia grew into disks, cushions, or plantlets apparently in the early macroscopic stage, all three bearing asexual plurilocular sporangia. Dammann (1930, p. 11) proved that the microscopic plantlets produced by zoöids from the unilocular sporangia bear plurilocular sporangia which are apparently asexual, and he obtained five successive generations from them, all of which in turn showed similar plurilocular sporangia. Thus, though alteration of generations has not been demonstrated in *Leathesia difformis* (L.) Aresch., the development of microscopic phases by both types of zoöids has been observed.

In *Mesogloia divaricata* (C. Ag.) Kütz., Hygen (1934, pp. 258–59) concludes from results obtained in cultures that zoöids from unilocular sporangia give rise to filamentous or disklike gametophytes, that reduplication of the latter occurs by macrogamete parthenogenesis, and that the union of macro- and microgametes starts the large sporophyte.

Nemalion multifidum (Weber and Mohr) J. Ag., according to Knight and Parke (1931, pp. 14, 23, and 24), is a "summer annual" that hibernates in a microscopic juvenile state. The earliest stage of the species is a horizontal branched or unbranched filament or a cell expanse (Chester 1896, p. 342, Figures 1–10; Lewis 1912a, p. 154; Cleland 1919, pp. 342–43, Plate XXIV, Figures 66–94).

It is not likely that the endophyte could be sufficiently protected by growing associated with the above microscopic winter phases consisting of filaments, disks, cushions or, at most, tuftlike plantlets. Also, the circumstance that the zoöids or carpospores are shed at about the same time as the zoöids of the endophyte further reduces the capacity of the microscopic phase of the host for sheltering the endophyte. It is found that the endophytic alga can go through its life history *in vitro*, and is able to exist independent of the host. No resting spores are found either in nature or in cultures. From these facts it appears probable that the endophyte hibernates in the form of juvenile plants which grow protected in minute crevices in rocks.

So far as is known, the summer phase of the endophyte is found only in a few hosts, and these have a cortex of radial assimilatory filaments embedded in a jelly. The compactness of the filaments and the firmness of the jelly may be determining factors in invasion, as would appear from the absence of the endophyte in *Chordaria flagelliformis* (Müller) J. Ag., which was common at Rocky Beach. Here the cortical filaments are more closely arranged and the gelatinous substance is firmer than in Mesogloia and Leathesia (Taylor 1937, pp. 139–40 and 143) or in

Nemalion. *Aegira virescens* (Carmichael) Setch. and Gard. was not found to be invaded, regardless of its very loose cortex and soft jelly, but it should be noted that the latter grows in exposed situations where the sea is quite rough.

Habit

The endophyte is hardly visible to the eye, but when the host becomes discolored and is dying, the cushions of the former appear as recognizable green specks or bands. Viewed with a hand lens, the areas occupied by the endophyte have a fuzzy appearance owing to the presence of setae. The interwoven filaments form a cushionlike thallus, which in Leathesia and Nemalion is punctiform with a diameter of 0.3–0.65 mm. In the strands of Mesogloia it extends into a complete or incomplete ring 0.7 mm. or less in width. In either case the thallus is firmly embedded in the gelatinous matrix present in the cortex of the hosts. Because fusions are common between filaments, the cushion is fairly compact, especially as growing in Leathesia (Plate I, Figures 7 and 10), and in Nemalion (Plate I, Figures 8 and 13), but in Mesogloia fusion takes place to a less extent. In cultures of the endophytes from all three hosts grown from the isolated endophytes, the degree of fusion is the same; the early stage consists of a regular or irregular disk of fused cells (Plate II, Figures 1–6), and later the mature thallus shows entangled filaments with considerable fusions (Plate I, Figure 3).

The tendency of branches to fuse is common to the three species of Ectochaete, though their habit is essentially filamentous. In *E. leptocaete* (Huber *l. c.*, p. 320, Plate XV, Figure 2) the disk is monostromatic with fused cells in the center and free filaments at the margins. *E. endophytum* develops into a polystromatic cushion with colorless cells in the interior (*l. c.*, pp. 322–24, Plate XV, Figures 12 and 13), thus the latter species exhibits greater pseudoparenchyma development than *E. Taylori* or *E. leptochaete* (*l. c.*, p. 325). In host relation *E. leptochaete* and *E. endophytum* differ from the species under discussion in that they enter the cell wall and live within it. In the later stages of *E. endophytum*, however, the cushion may break through and become free.

Setae

Hyaline setae not separated by a wall from the cell below and typically not twisted nor having undulating walls are characteristic of Ectochaete. In the present species they are always numerous when it is growing in its natural habitat, and they are firm and conspicuous, reach-

ing a length within 0.8 mm. and a diameter usually of 2.66 microns. *E. endophytum* is described as bearing similar setae, but they are not numerous; they have been found to develop in large numbers, however, in cultures (l. c., pp. 322 and 324). In *E. leptochaete* the setae are extremely delicate. Feldman (1937, p. 181) used iodized hydriodic acid to detect them and mentioned that they are seen with difficulty when examined directly; in the latter species the seta exhibits a constriction at the point where it emerges from the host. The seta has colorless transparent cytoplasm but has no nucleus. In *E. Taylori* it may break near the tapering apex, and if so it remains open and devoid of contents. When a young seta breaks, proliferation may occur, and if it does, the old part looks like a sheath (Plate I, Figure 12). Similar false sheaths have been demonstrated in *Acrochaete repens* Pringsh. and *Bolbocoleon piliferum* Pringsh. (Huber 1892a, p. 329).

Cell size and structure

The cell diameter varies from 8 to 15 microns and may reach 18 microns. The length is 1 to 3 times the diameter and occasionally 5 times. In *E. endophytum* the diameter is 8–20 microns and may be as great as 30 microns, the length being 1–3 times or as great as 5 times. *E. leptochaete* has a cell diameter of 5–10 microns, sometimes attaining 15 microns, and the length is 1–3 times. The chloroplasts of all three species are platelike or occasionally perforate; their form is that of an incomplete girdle; they are either homogeneous or, owing to the presence of starch grains, granular. *E. leptochaete* has fewer pyrenoids than the other two. In *E. Taylori* the single nucleus in a cell becomes visible after having been stained with acetocarmine.

Gametangia

A large number of nonsetigerous cells are transformed into gametangia. They are usually sac-shaped, because of the presence of a wide dorsal neck. When the gametangia are more or less deeply embedded

---

PLATE I

E. Taylori. 1, 2, 3, 4, 5, 12 from Hesogloia. 6, 7, 9, 10 from Leathesia. 8, 11, 13 from Nemalion. (1) Germling showing chloroplasts, 1000 ×. (2) Branch of horizontal system with terminal seta, 450 ×. (3) Gametangia and fusion of filaments (culture), 528 ×. (4) Erect branches in the place of setae (culture), 400 ×. (5) Germling attached to Mesogloia filament and showing growth at an angle to the long axis of zoöid, 450 ×. (6) Gametangia with short necks, 875 ×. (7 and 10) Fused filaments among host filaments, 450 ×. (8) Disco-like germling on surface of host, 400 ×. (9) Gametangia with long necks among host filaments, 450 ×. (11) Germlings, note gametangium, 400 ×. (12) Cell bearing seta with false-sheath, 750 ×. (13) Germling on surface of host, 400 ×.

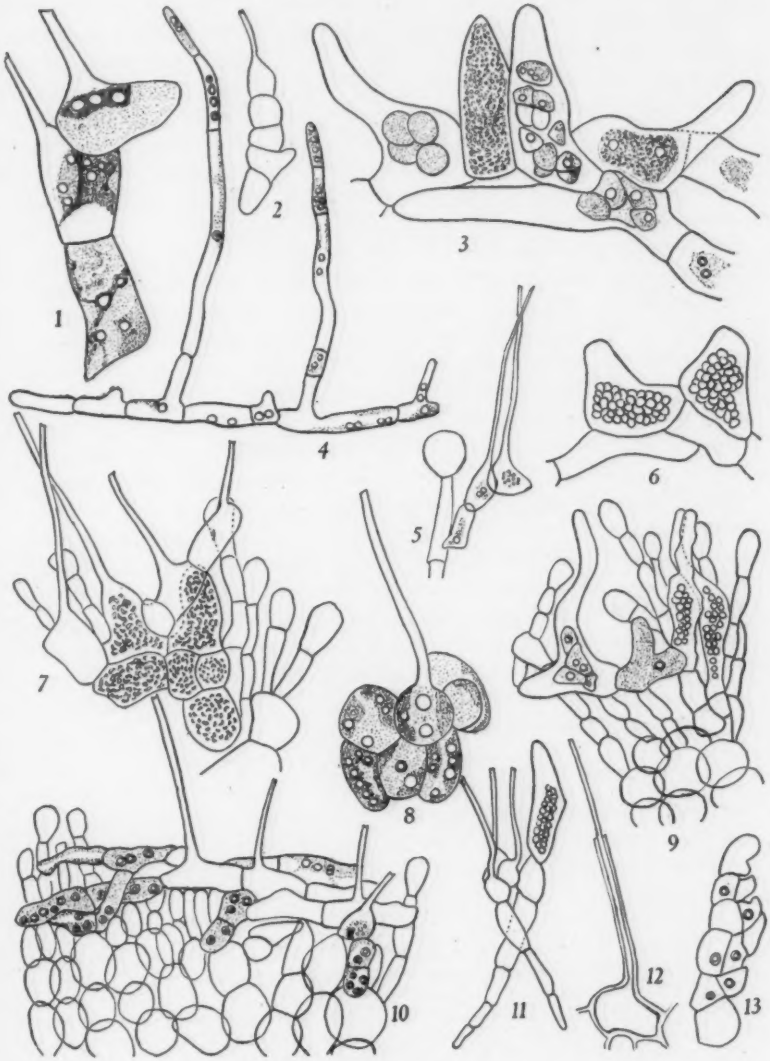


PLATE I

the neck may be two to five times the width of the cell proper, and, further, it may be sinuous (Plate I, Figure 9), but if the gametangia are near the surface of the host the neck is straight and short, being ⅓–2 times the diameter of the cell (Plate I, Figure 6). Without regard to the length the base of the neck agrees in width with that of the cell; it tapers slightly towards the apex, where it is quite rounded and is 2.66–12 microns across. The gametes are numerous, and usually about 30–60; they are biflagellate, ovoid or, sometimes, elliptic; their diameter varies from 1.76 microns to 4.4 microns and may at times be 6.2 microns; in length they are 3.52 microns to 6.2 microns and occasionally reach 8.8 microns. The flagella are 1½ to 2 times the length of the gamete. There is a choroplast with a pyrenoid; an eye spot is present (Plate II, Figures 8*c* and 9*a*). The gametes are expelled suddenly and with considerable force, and in this respect the present alga resembles *Ochlochaete ferox* Huber (1892b, p. 293). Emission of gametes may be easily observed upon exposing sections of the host with fruiting thalli of the endophyte in the illuminated field of the microscope for a few minutes. The contents of the gametangia are seen to stream quickly upwards through the neck and are expelled in a mass to a distance of about 0.4–0.8 mm. beyond the surface of the host, but not beyond the projecting setae. After this they either instantaneously swim, with a rapid motion, in the direction in which they were shot out, or they are motionless for some seconds and gradually begin to swim severally in various directions—avoiding the host, however. At times the swarmers under the cover glass rotated rapidly (Plate II, 9*b*), remaining in one spot as though pivoted on the beak.

The gametes are either parthenogenetic or they unite in pairs. The fusing gametes were found to be equal in size. At times, a pair after joining by their beaks spin rapidly in one spot just as single gametes may do. At the end of a few minutes the spinning stops and the zygote, which is visibly larger than a gamete, moves away with a slow motion. Gametes are more or less positively phototactic, while the zygote is nega-

---

PLATE II

Ectochaete Taylori. 1, 2, 3, 4, 5, 6 (cultures in 0.375 Detmer sol.), from Mesogloia. 7 from Mesogloia kept in specially aerated sea water. 8 from Nemalion. 9, 10 from Mesogloia. (1) Germination at an angle to the long axis of zoöid, 715 ×. (2, 3, 4) Stages in growth of irregular disklike germlings, 715 ×. (5) Regular disklike germling, 715 ×. (6) Later stage than 5, but prior to development of erect branches, 275 ×. (7) Germling with abnormal setigerous cell, 450 ×. (8) 1000 ×: (*a*) fusing gametes. (*b*) Zygote showing two stigmata. (*c*) gamete. (9) 1000 ×: (*a*) gamete. (*b*) Spinning gamete. (*c*) Fusing gametes. (10) Zygotes. (*a*) 1200 ×. (*b*) Shows two stigmata, 1500 ×. (*c, d*) Show two plastids each, 1000 ×. 8, 9, 10, except 9 *b*, fixed in iodine.

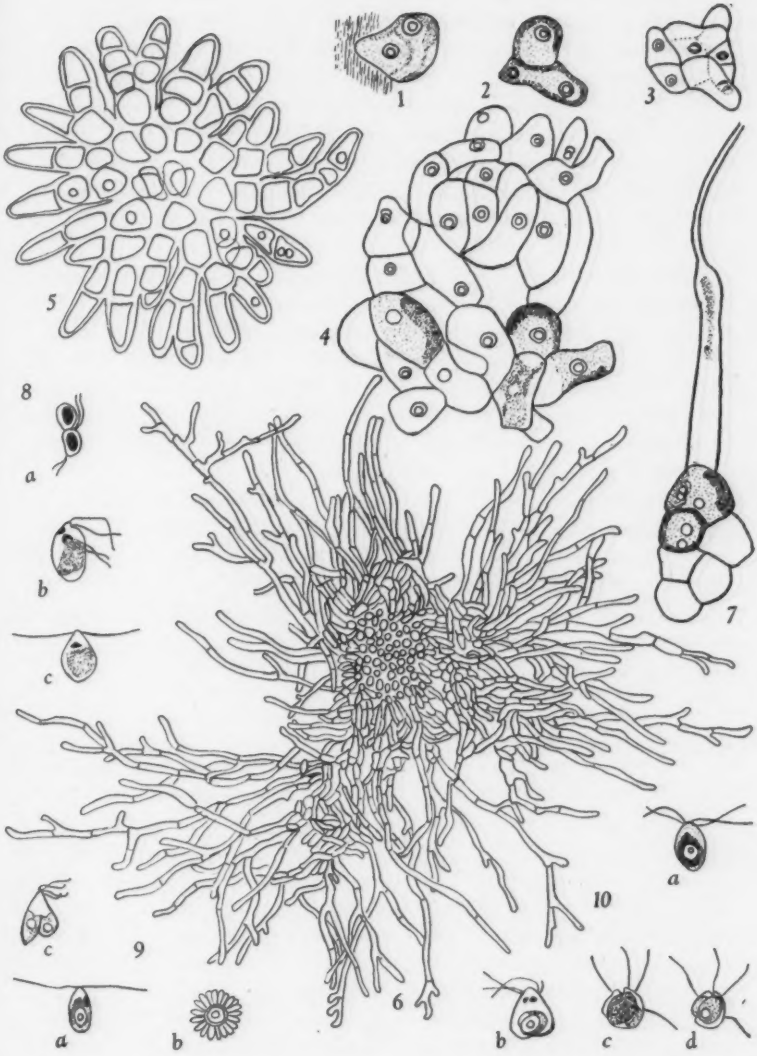


PLATE II

tively phototactic and is soon lost to sight, as it becomes hidden in the host tissue. In some instances fusing pairs of gametes stopped spinning for a time and it was possible to observe that fusion proceeded from the beak to the posterior end. Zygotes were fixed with a 2 per cent solution of I_2. Fusion appears to take place rapidly as a rule, for it is difficult to find examples of intermediate stages of the process (Plate II, Figures 8*a* and 9*c*). Zygotes seen were invariably near the host tissue and about to enter it. In some cases paired chloroplasts, eye spots, and pyrenoids could be recognized in the zygotes (Plate II, Figures 8*b*, 10*b*, 10*c*, and 10*d*).

Sexual reproduction has not been observed in *E. leptochaete* and *E. endophytum*, but germination of biciliate zoöids is known for the former, while in the latter, zoöids have not been seen after liberation, though germination stages were obtained. Huber (1892b, Plate VI, Figure 6) states that the zoospores of *E. leptochaete* are 4–5 microns in diameter and ovoid or round in shape; the flagella appear from the figure to be nearly 3 times the length of the zoospore. This species lacks an eye spot (l. c., p. 325).

The sporangia of *E. leptochaete* and *E. endophytum* are short-necked and resemble the short necked type of gametangium found in *E. Taylori*. In *E. leptochaete* the length of the neck is one-third of the width of the cell (l. c., Plate XIV, Figure 5) and in *E. endophytum* (Möbius 1891, Figures 5 and 7) it is the same as the cell width. Huber observed the liberation of zoospores in *E. leptochaete* but did not relate anything special about the process.

Germination

The gamete or zygote settles on its beak, surrounds itself with a cell wall, and begins to elongate at the posterior end, at an angle to the principle axis, and goes on to develop a filament (Plate I, Figures 1, 5, and 11). In cultures, in the absence of the host, as well as when the alga in nature germinates superficially on its host, the zoöid grows in the same way but usually in several directions perpendicular to the long axis, and thereby forms a disk or irregular expanse of cells (Plate I, Figures 8 and 13; Plate II, Figures 1–6). This type of germination is characteristic of the epiphytic series of prostrate Chaetophoraceae (l. c., p. 350), and it is interesting to find that this species of Ectochaete, though endophytic, still retains a relatively simple form of germination.

In *E. leptochaete* and *E. endophytum* the zoöid, after coming to rest and forming a wall around itself, sends a tube from its anterior end into the host wall. Once within, the germination tube expands in a plane

perpendicular to itself and gives rise to an elongate cell into which the contents of the zoöid pass. *E. endophytum* may sometimes vary slightly from the above process, and its zoöid then grows into a large cell provided with a seta; but it is later that the germination tube grows out from the anterior end.

Germination in the Chaetophoraceae appears to be a somewhat variable character. On the one hand it permits flexibility in the relation of algal habit to host or substratum and on the other it probably helps in bringing about speciation.

The variation in germination within the genus Ectochaete may be compared to that in Stigeoclonium which has two kinds of epiphytic germination. Species like *S. flabelliferum* and *S. variabile* (Berthold 1878, pp. 199–200, Taf. I, Figures 16 and 18) germinate by growth along the principal axis, from the posterior end of the zoöid, that is, in a direction opposite to that obtaining in typical endophytic germination. An erect filament thus is developed, and from its basal cell the horizontal system of branches takes its origin. *S. lubricum* is representative of the second kind of epiphytic germination found here, and its zoöids grow in several directions perpendicular to the principal axis, thus forming the creeping system from which the erect system arises (l. c., pp. 200–201, Taf. I, Figure 9).

Differences in germination between the species of a genus is present also in *Endoderma* Lagerheim. *E. perforans* Huber is one that produces a germination tube at the anterior end of the zoöid. *E. majus* Feldman (1937, pp. 182–83) and *E. testarum* Kylin (1935a, p. 199) are among species that are epiphytic in germination while they are in habit endophytic and shell inhabiting respectively.

Proof of similar variation within a species is afforded by *Phaeophila dendroides* (Crouan) Batters. On certain hosts it is endophytic and on others epiphytic in germination (Huber 1892b, p. 330, Plate XVI, Figures 4 and 7) and in habit.

The above account and comparison go to show that on the grounds of habit, structure, and host relationship *Ectochaete Taylori* appears to be a well-circumscribed species and is distinct from the two previously known species of the genus.

Homology and Function of Setae

In cultures of the alga grown in 0.375 Detmer's solution, it was noticed that the swarmers developed into minute, more or less regular disks (Plate II, Figures 1–5), the cells of which lacked hairs. As the disks grew older they produced horizontal branches in many directions

(Plate II, Figure 6) and still later erect filaments arose from the creeping filaments (Plate I, Figure 4). The former differ from the latter in being slightly narrower, in consisting of only a few very long cells, the chloroplasts of which are rather poorly developed, and in usually lacking a cross wall between the basal cell and the parent filament. When these cultures were transferred back to sea water without additional nutrients, it was found that setae arose from the horizontal system of filaments and also occasionally from the erect filaments formed earlier in these cultures, but it was noteworthy that new erect filaments did not arise. The erect filaments seen in cultures of *E. Taylori* which are comparable to those obtained by Huber (1892a, p. 333, Figure 7) in *Phaeophila dendroides* (Crouan) Batters (*Phaeophila floridearum* Hauck) bear out further the theory (Huber 1892a, p. 222; 1892b, pp. 344–45) that hairs and setae in the procumbent Chaetophoraceae are reduced erect branches of the heterotrichous habit.

Another fact regarding the development of setae in the group is observable in the alga under discussion. It is noticed that both in nature and in cultures the apex of a horizontal filament may sometimes end in a seta which is thus a continuation of the long axis of the filament concerned (Plate I, Figure 2). Attention has been directed to a similar origin of the solid setae of *Gonatoblaste rostrata* Huber (Fritsch 1929, p. 258, Figures G and H). It therefore appears that in the procumbent Chaetophoraceae the reduction of branches and development of setae may occur in the horizontal system as well.

That intact setae with living cytoplasm may possibly function in absorption is suggested by their reappearance on bringing cultures from 0.375 Detmer solution in sea water to plain sea water. In this connection it may be mentioned that Hygen (1934, p. 248) found that, under unfavorable conditions, especially those caused by nutrient deficiency, hair development increased in the microscopic phases of *Nemacystus divaricatus* (Ag) Kuck., and Huber described greater hair development under similar conditions for *Stigeoclonium* (1892a, p. 323–24).

Taxonomic Position

Since *Ectochaete* (Huber) Wille has been segregated from *Endoderma* Lagerheim it requires to be given a place with reference to the genera of procumbent Chaetophoraceae (Huber 1892b, pp. 352–3). Its natural position would seem to be between *Bolbocoleon* Pringsh. and *Endoderma* Lagerheim. The former is characterized by small, bulbous, setigerous cells that represent erect branches, whereas in *Ectochaete* (Huber) Wille the erect system of branches is further modified in that

the setae arise directly from the horizontal filaments. In *Endoderma* Lagerheim the reduction is almost complete and setae are absent.

The reduced number of setae in *Ectochaete endophytum* and *E. leptochaete* and their typical endophytic germination signify that both species are probably nearer to Endoderma than to Bolbocoleon. The simple monostromatic thallus of *Ectochaete leptochaete* would seem to indicate that the latter is the species nearest the level of Endoderma. In contrast, the presence of conspicuous setae, the method of germination, and the general habit show that *Ectochaete Taylori* is the least specialized of the related species and is on the whole more representative of the Bolbocoleon level of development in the group. *Phaeophila* Hauck and *Ochlochaete* Thwaites have a degree of reduction of the erect system of branches similar to Ectochaete, but they differ from the latter in other important respects. Ochlochaete forms definite disks of closely fused cells and is thus more strongly dorsiventral than Ectochaete; in detail, the former differs from the latter in having but one to two pyrenoids in the cell and in its zoospores, each of which is provided with four flagella. Unlike Ectochaete, Phaeophila ordinarily lacks fusions between filaments, and a cell may frequently bear two setae. Phaeophila may be readily distinguished from Ectochaete: its setae are twisted or have undulating walls, the emission tube of its sporangium is narrow, and its zoospores are four-flagellate. The differentiation of the chloroplast into thicker minute disklike areas, referred to by Huber (1892b, p. 328) was not evident in *Phaeophila dendroides* obtained at Woods Hole; nevertheless, its identity was clear when based on the above characters.

The writer wishes to express her thanks to Dr. Wm. R. Taylor for the guidance received in the course of this study, to Dr. Alma G. Stokey for her invaluable suggestions, and to Professor H. H. Bartlett for extending his help in writing the Latin description of the species.

LITERATURE CITED

BERTHOLD, G., 1878. Untersuchungen über die Verzweigung einiger Süsswasseralgen. *Nov. Act. Leop. Carol. Akad.*, **40**: 169–230.

CHESTER, G. D., 1896. Notes concerning the development of Nemalion multifidum. *Bot. Gaz.*, **21**: 340–347.

CLELAND, R. E., 1919. The cytology and life-history of Nemalion multifidum Ag. *Ann. Bot.*, **33**: 323–351.

DAMMANN, H., 1930. Entwicklungsgeschichtliche und zytologische Untersuchungen an Helgoländer Meersalgen. *Wiss. Meeresunters., N. F., Abt. Helgoland*, **18** (i, 4): 1–36.

FELDMANN, J., 1937. Les algues marines de la côte des Albères II, Chlorophyceae. *Rev. Algol.*, **9** (3–4): 173–241.

FRITSCH, F. E., 1935. The Structure and Reproduction of the Algae. Cambridge Univ. Press.

HUBER, M. J., 1892a. Observations sur la valeur morphologique et histologique des poils et des soies dans les Chaetophorées. *Jour. de Bot.*, **6**: 321–41.

HUBER, M. J., 1892b. Contributions à la connaissance des Chaetophorées épiphytes et endophytes et de leurs affinités. *Ann. Sci. Nat., Bot. VII*, **16**: 265–359.

HYGEN, G., 1934. Über den Lebenzyklus und die Entwicklungsgeschichte der Phaeosporeen. Versuche an Nemacystus (C. Ag) Kuck. *Nyt Mag. Naturvidensk.*, **74**: 187–268.

KNIGHT, M., AND PARKE, M. W., 1931. Manx Algae. *Mem. Liverpool Mar. Biol. Corp.*, **30**: 1–155.

KYLIN, H., 1933. Über die Entwicklungsgeschichte der Phaeophyceen. *Lunds Univ. Arsskr.*, N. F., Avd. 2, **29** (7): 1–95.

KYLIN, H., 1933. Über einige kalkbohrende Chlorophyceen. *Kungl. Fysiogr. Sallsk. i Lund, Forhandl.*, **5** (19): 1–19.

LEWIS, I. F., 1912a. The germination of the spore of Nemalion multifidum. *Science* N. Ser., **35** (891): 154.

MÖBIUS, M., 1891. Conspectus algarum endophytarum. *La Notarisia* **6** (26): 192.

SAUVAGEAU, C., 1925. Sur le développement d'une algue phéosporée, Leathesia difformis Aresch. *Comptes Rendus Acad. Sci. Paris*, **180**: 1632–35.

TAYLOR, W. R., 1937. Marine Algae of the Northeastern Coast of North America. Univ. of Michigan, Ann Arbor, Mich.

WILLE, N., 1909. Conjugatae und Chlorophyceae. In A. Engler und K. Prantl, *Die natürlichen Pflanzenfamilien*, 1. *Anfl., Nachtr. z.*, I, **2**: 1–97.

THE EFFECT OF ENVIRONMENTAL FACTORS ON THE SPERM CYCLE OF TRITURUS VIRIDESCENS[1]

JOHN D. IFFT

(Osborn Zoological Laboratory, Yale University, New Haven)

Introduction

The problem of vertebrate cyclic gametogenesis and its relation to the environment has been the subject of considerable experimental investigation (for reviews, see Bissonnette, 1936, 1937; Marshall, 1936, Rowan, 1938a, b). However, the reproductive cycles of relatively few species of Amphibia have been experimentally studied.

In South African clawed toads, *Xenopus laevus*, Hogben, Charles, and Slome (1931) reported the ovaries of blinded animals to be in a subnormal condition while ovarian regression did not occur in animals in constant light. The so-called "captivity effect," resulting in the loss of sexual activity in Xenopus, was noted by Shapiro and Zwarenstein (1933) and Shapiro (1936) when the animals were kept in the laboratory. Alexander and Bellerby (1935) concluded that the gonads did not degenerate in the laboratory if sufficient food was available and the normal periodicity occurring in nature disappeared. Later (1938) these authors found ovarian regression was induced by decreasing the water volume of the tanks and concluded the seasonal decrease of water volume in Xenopus' ponds was a contributory cause of the ovarian cycle. Bles (1905) had considered this phenomenon earlier. Light was not found essential for reproductive activity (Bellerby, 1938). Hypophysectomy led to gonad regression in both sexes (Bellerby and Hogben, 1938).

Galgano (1931, 1932, 1934, 1936) in a series of studies on *Rana esculenta* found that animals in the laboratory at 18°–24° C. in December or January, without additional light, resumed spermatogenesis when normally there is no gonadal activity at that time. He concluded (1936), "Dalle osservazioni eseguite e sopra succintamente esposte, trassi la convinzione che la spermatogenesi in Rana esculenta fosse potenzialmente per fattori intrinseci di tipo continuo e che soltanto la causa occasionale esterna della temperatura insufficiente determinasse la sua interruzione nella cattiva stagione." Galgano observed that the phase of the

[1] This paper is part of a dissertation presented to the Faculty of the Graduate School of Yale University in candidacy for the degree of Doctor of Philosophy.

sperm cycle of animals collected from different localities in Europe and Africa could be correlated directly with the varying temperatures of the diverse localities; Witschi (1924), however, found that in certain races of *R. temporaria* the spermatogenic cycle had become hereditary and was not modified by climatic changes. Shapiro (1937) states that constant additional light of 300 candle power from February 26 to April 12 did not show whether light had any effect on mating and oviposition in *R. temporaria*. Rowan (1938b) and Bullough (1939) reported a personal communication from Spaul and Gladwell that *R. temporaria* reacted by gonad growth, gametogenesis and spawning when subjected to fourteen hours of electric light per day during the winter with a temperature of 20° C. The newt, *Triton cristatus*, also bred under these conditions, while gravid females of the viviparous salamander, *Salamandra maculosa*, gave birth to young when exposed to lengthy illumination, but not otherwise.

In view of the relative incompleteness of the knowledge of this problem in Amphibia, the present study was undertaken to discover if the external environment influenced the sperm cycle of the salamander, *Triturus viridescens viridescens*.

The author wishes to thank Professors J. S. Nicholas and Daniel Merriman under whose direction the study was made, Mr. D. J. Zinn for assistance in collecting animals, and the Choate School where part of the work was done while the author held the Choate Research Fellowship in Biology.

Materials and Methods

Adult male *Triturus viridescens viridescens* of approximately the same size and from the same pond in Hamden, Connecticut, were used in all the experiments.

Conditions in the experimental tanks were kept as uniform as possible except for the variable being tested; the water volume was the same for each animal and all animals were fed a similar diet of ground beef. In most of the lighting experiments the foot candles of illumination were measured five inches below the water surface by a submarine photometer (Zinn and Ifft, 1941); the illumination for each specific group is given in the section on the experiments. Animals kept at low temperatures were kept in an electric refrigerator with a recording thermometer to show the temperature variations.

The effects of the experimental conditions on the spermatogenic cycle were determined by the following methods:

(1) The most anterior lobe of the testis of the right side was removed, fixed in Bouin's, sectioned at 25 microns and stained with Delafield's haemotoxylin. Sections of 25 microns in thickness were suitable for the study, since the long axes of the various cysts range approximately from 100 microns to 500 microns. Planimeter measurements were made from microprojection drawings ($\times$ 10) of every tenth section; primary and secondary spermatogonia were measured together as were the primary and secondary spermatocytes. The percentage of the total area occupied by each spermatogenic stage was then determined. Care was taken to check each measured area with higher magnification ($\times$ 880) to make certain of the spermatogenic stage.

(2) A second method was to make planimeter measurements of the center section of each testis.

(3) A third method was to separate by dissection the sperm areas from the non-sperm areas and weigh each. This separation can be easily made at certain stages of the cycle as the gross appearance (Adams, 1940) of the two areas is distinct and a definite plane exists between them.

Normal Sperm Cycle of Triturus viridescens

The spermatogenetic cycle of *Triturus viridescens* has been described by Hilsman (1932a, b) and Adams (1940). The normal cycle studied in the course of this investigation agrees with that reported by Adams. At the time of the spring breeding season in late April the testes consist of spermatogonial vesicles clearly divided from the region of mature sperm cysts and a varying number of evacuated cysts from the "false" breeding season of the previous autumn. The discharge of sperm continues into June. During the summer active gametogenesis is in progress, and all stages of germ cells may be found until September, when the testes consist chiefly of spermatogonia and mature sperm. At this time the "false" breeding season begins, and varying amounts of sperm are discharged, leaving the evacuated cysts which remain until spring. The females do not ovulate at this time. During the winter months the testes are dormant with the same appearance as that described for the beginning of the spring breeding season. Adams concludes that normally there is one annual cycle of spermatogenesis in *Triturus viridescens* and this is confirmed by the present investigation, but does not agree with Hilsman who states that there are two independent spermatogenetic cycles corresponding with the two "breeding" seasons.

Experimental Results

Experiment I.—The first experiments were designed to determine if light or temperature or combinations of these factors would have any effect during the period of active gametogenesis. Sixty animals were collected on July 27, 1939, and divided into six groups of ten each. For comparative purposes there was added a seventh group which was collected on September 4 and killed on that date.

(1) Ten control animals, normal daylight through glass, temperature, 20°–24° C.

(2) Ten animals with constant light from a fluorescent lamp delivering 32 foot candles at the surface, 13 f. c. at the tank bottom; temperature, 20°–24° C. Two of these animals died from a fungus infection.

(3) Ten animals, bilaterally enucleated (entire ocular globe removed), constant light and temperature as in group 2.

(4) Ten animals in constant darkness; temperature, 20°–24° C. Six of these animals died from a fungus infection.

(5) Ten animals in constant darkness; temperature, 11°–15° C. until September 10 and then lowered to 6°–7.5° C. until September 28. (A change in the refrigeration unit caused this lowering of temperature.)

(6) Ten animals in constant light from a fluorescent lamp delivering 32 f. c. at tank surface and 16 f. c. at bottom; temperature as in group 5.

(7) Five animals collected on September 4 and killed on that date.

Except for group 7 all of these animals were killed on September 28 after a period of 63 days. The testes were sectioned with planimeter measurements being made of every tenth section.

An analysis of the results (Figure 1) indicates: (1) At room temperature all animals showed the same degree of spermiogenesis. That

---

Plate I

Sg = Spermatogonia; Sc = spermatocytes; St = spermatids; S = sperm; DgCy = degenerating cysts.

All figures are sections of testes from animals in Experiment I.

Figure 1. From an animal kept in constant light at room temperatures (20°–24° C.) (Group 2). Mag. about × 10.

Figure 2. Control animal; daylight through glass, room temperatures (Group 1). Mag. about × 10.

Figure 3. From an animal kept in constant light at low temperatures (12° C.) (Group 6). Mag. about × 18.

Figure 4. From Group 5 under similar conditions as in Figure 3 except in constant darkness. Mag. about × 18.

Figure 5. In constant darkness, room temperatures (Group 4). Mag. about × 10.

Figure 6. In constant light, room temperatures, with both eyes removed (Group 3). Mag. about × 10.

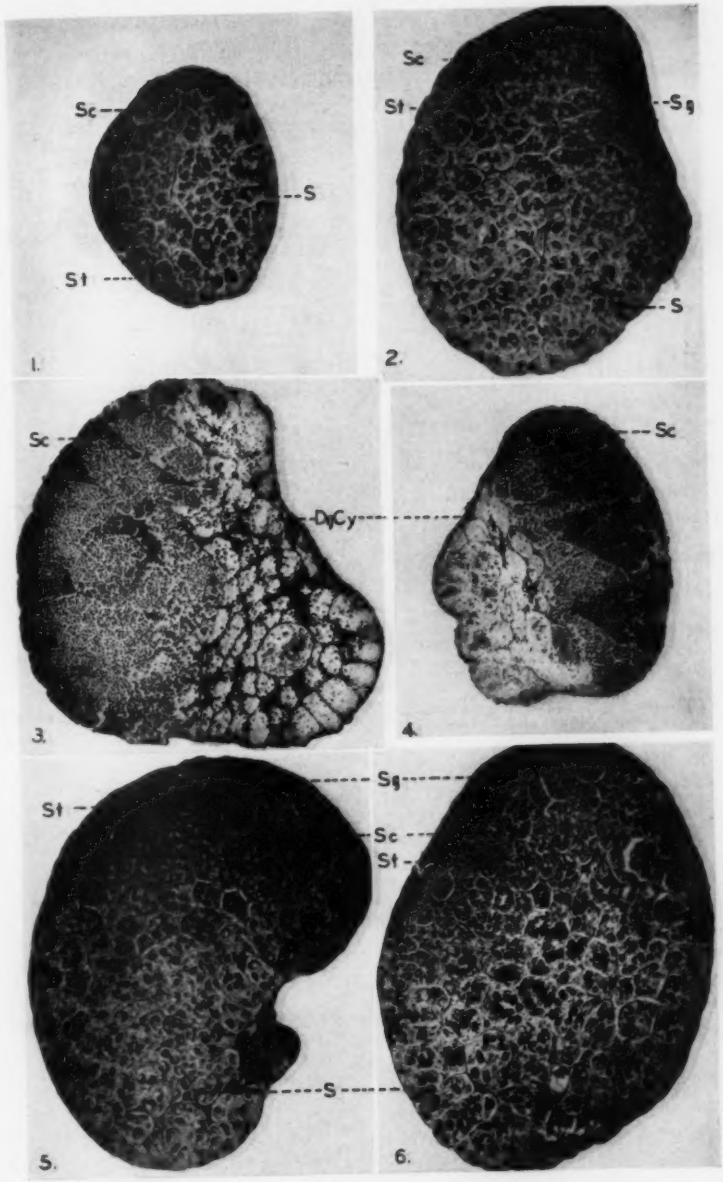


PLATE I

is, approximately equal percentages of mature sperm were found in each of the room-temperature groups irrespective of lighting conditions. The differences in these average percentages were no greater than the differ-

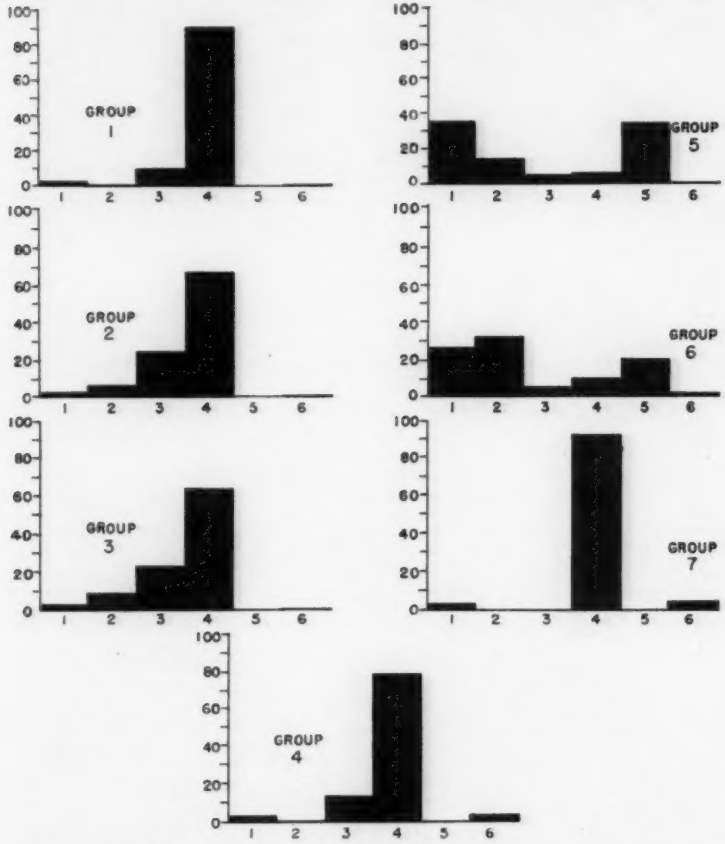


FIGURE 1. Results of Experiment 1 showing the average percentage of the testis occupied by the various germ cells. Refer to the text for the experimental conditions of the groups. Key: 1, Spermatogonia. 2, Spermatocytes. 3, Spermatids. 4. Sperm. 5, Degenerating cysts. 6, Non-germinal.

ences within the individual groups. It is evident, therefore, that light was not necessary for normal spermiogenesis, nor did constant light produce a greater degree of spermiogenesis. In the control group there was considerable variation, ranging from 69.3 per cent sperm to 96.9

per cent. Some spermatids were still present so that the spermiogenesis was not complete except in two of the animals. The analysis of the five normal animals (Figure 1, group 7) collected on September 4 showed that spermiogenesis was more rapid in nature as there were no spermatocytes or spermatids present; this was probably due to the fact that the water-temperatures in the pond (range of 25°–30.5° C. from June 28 to September 4) were higher than those in the laboratory experiments. (2) At low temperatures (groups 5 and 6) spermiogenesis had ceased since a high percentage of spermatogonia and spermatocytes was present while a low percentage of sperm areas was found. These sperm were noted, upon histological examination, to be not newly formed but were undischarged ones remaining from the spring breeding season. Undischarged sperm of this type have a characteristic appearance, the sperm being "loosely" arranged in the cysts in contrast to the compactness of newly formed sperm. The cysts are often partially discharged and fragmented sperm are sometimes found. There were no significant differences between the animals in constant light and those in the dark. A noticeable low-temperature effect was the extensive areas of cysts in which the germ cells were degenerating. Two types of cells were involved in this degeneration; certain phases of the spermatocytes were completely degenerated leaving only empty cysts, while the spermatids had typically pycnotic nuclei. The cysts did not collapse as occurs following normal evacuation. The following measurements of the long axes of various types of cysts indicate the approximate average sizes:

Normal newly formed sperm cysts	440 microns
Normal mature sperm cysts	300 microns
Normal spermatid cysts	360 microns
Normal spermatocyte cysts	240–320 microns
Normal evacuated cysts	0–160 microns
Degenerating spermatid cysts	360 microns
Degenerating spermatocyte cysts	260 microns

The cysts containing the degenerating cells maintained the normal size for cysts of that type. Spermatogonia were not affected by the low temperatures in this experiment.

The differences between the testes' weights of the various groups give additional evidence of the inhibitory effect of low temperatures. The most anterior lobe on the left side of each animal was weighed and the percentage of the body weight was determined. The average percentages of the body weights of each group are given below (Figure 2).

The differences between the sperm areas in the various experimental groups were analyzed by Fisher's (1938) method for determining the significance of differences between small samples; Table I summarizes

the results. The low-temperature groups are not comparable with the others because of the previously mentioned fact that the sperm present were undischarged from the previous cycle and, therefore, were not

FIGURE 2. Per cent of the body weights formed by the testes in the animals in Experiment 1.

indicative of spermiogenesis occurring at the time of the experiment. Statistically, constant light at room-temperatures apparently inhibited spermiogenesis and while this may be true, obviously it is not a factor in the normal cycle.

To summarize the results of this experiment, it is clear that temperature and not light is the important external environmental factor con-

TABLE I

Statistical Analysis of Experiment 1

	Control	Constant light, 20°–24° C.	Eyeless, constant light, 20°–24° C.	Constant darkness, 20°–24° C.	Constant darkness, low temperature	Constant light, low temperature	Normals
Control		P = .05–.02 S	P = .01–	P = .3–.2	N.C.	N.C.	P = .3–.2 N.S.
Constant light, 20°–24° C.	P = .05–.02 S		P = .5–.4 N.S.	P = .05–.02 S	N.C.	N.C.	P = .01– S
Eyeless, constant light, 20°–24° C.	P = .01– S	P = .5–.4 N.S.		P = .05–.02 S	N.C.	N.C.	P = .01– S
Constant darkness, 20°–24° C.	P = .3–.2 N.S.	P = .05–.02 S	P = .05–.02 S		N.C.	N.C.	P = .01– S
Constant darkness, low temperature	N.C.	N.C.	N.C.	N.C.		P = .1–.05 N.S.	N.C.
Constant light, low temperature	N.C.	N.C.	N.C.	N.C.	P = .1–.05 N.S.		N.C.
Normals	P = .3–.2 N.S.	P = .01– S	P = .01– S	P = .01– S	N.C.	N.C.	

P = probability.
S = significant.
N.C. = not comparable (see text).
N.S. = not significant.

trolling spermiogenesis. All of the animals kept at room temperatures (20°–24° C.) showed approximately equal degrees of sperm formation irrespective of lighting conditions, while in the animals kept at low temperatures spermiogenesis was not only stopped but large percentages of spermatocytes and spermatids degenerated.

Experiment II.—A second set of experiments was designed to test temperature effects at an earlier phase in the spermatogenic period and also to find if food supply influenced the cycle.

Forty-seven animals collected on May 2, 1940, were divided into six groups, as follows:

(1) Eight animals, killed at the beginning of the experiment on May 2. Temperature of pond at collection date, 15° C.

(2) Seven animals, without food; normal light, at room temperature (20°–25° C.).

(3) Seven animals, fed regularly; other conditions as in group 2.

(4) Ten animals, fed regularly; constant light from a fluorescent lamp delivering 32 f. c. at tank surface and 16 f. c. at bottom; at temperature of 5°–8° C.

(5) Eight animals, constant dark with other conditions as in group 4.

(6) Seven animals, as in group 5, except for temperature at 1.5°–3.5° C.

All of the animals (except group 1) were killed on July 1; Figure 3 shows the results of this experiment.

An analysis of these results again indicated the importance of temperature as a controlling factor in spermatogenesis. At the beginning of the experiment, as the normal animals (group 1) show, the testes were discharging sperm and a high percentage (51 per cent) of evacuated cysts were present. The remainder of the testes consisted of spermatogonia (25.4 per cent) and undischarged mature sperm (23.4 per cent). All of the animals (groups 4, 5, and 6) at low temperatures were essentially in the same stage on July 1 as the animals at the beginning of the experiment. The sperm present were undischarged mature sperm, not newly formed ones. This indicated again that low temperatures inhibited spermatogenesis irrespective of light conditions as none of the cell type percentages were greatly different whether the animals were in light or darkness. There were no significant differences between the animals at 5°–8° C., constant darkness, and those at 1.5°–3.5° C., constant darkness. None of the low-temperature animals had degenerating cysts, and since no spermatocytes or spermatids were found at this phase of the cycle, the observation that cold affects only these types of cells was confirmed.

The animals kept at room temperature (groups 2 and 3) underwent active spermatogenesis with the production of spermatocytes, spermatids and new sperm. The evacuated cysts completely disappeared. There was little difference between the fed and non-fed animals, a fact which indicated that the food supply had no influence on the germ-cell cycle in this experiment.

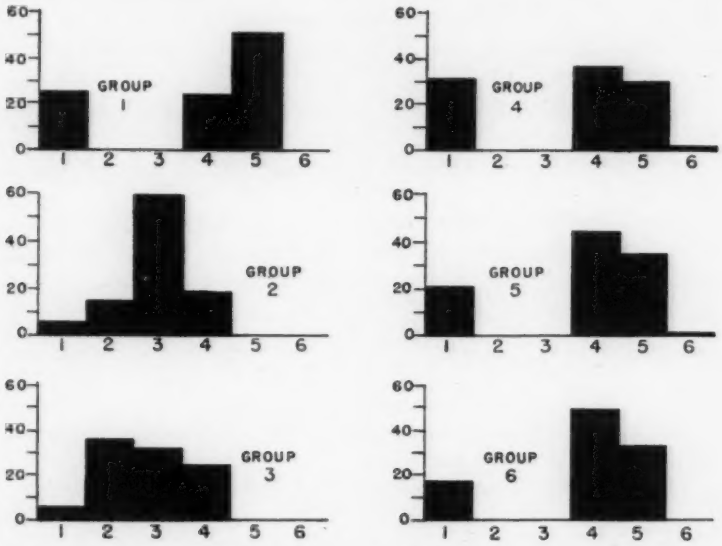


FIGURE 3. Results of Experiment 2 showing the average percentage of the testis occupied by the various germ cells. Refer to the text for the experimental conditions of the groups. Key: 1, Spermatogonia. 2, Spermatocytes. 3, Spermatids. 4, Sperm. 5, Evacuated cysts. 6, Non-germinal.

Experiment III.—The next series of experiments were made during the winter months to find if a steadily increasing light period would have any influence on either gametogenesis or on sperm discharge with temperatures at approximately the threshold value for germinal activity. The threshold temperature was considered to approximate 15° C. as temperatures just below were inhibitory, while under natural conditions breeding begins at this temperature. The 59 newts collected on November 3, 1939, were kept at temperatures averaging 15° C. until February 7 when they were placed in the experimental groups listed below. An additional 21 animals collected on February 6 were included in the experiments.

(1) Five animals collected on November 3, kept at 14°–16° C. until February 7 when they were killed.

(2) Five animals collected on February 6, killed on February 7.

(3) Nine animals collected on November 3 under same conditions as group 1 until killed on March 7. Light through glass during midday 43 f. c.

(4) Ten animals collected on November 3, temperature, 14°–16° C.; light increasing 15 minutes per day from 10 hours on February 7 to 16.5 hours on March 7 with an intensity of 21 f. c.

(5) Eight animals collected on November 3 kept under constant light, 16 f. c. from February 7 to March 7; temperature, 14°–16° C.

(6) Ten animals collected on November 3 kept in constant darkness from February 7 to March 7; temperature, 15°–17.5° C.

(7) Eight animals collected on November 3 kept in constant darkness from February 7 to March 7; temperature, 5°–8° C.

(8) Nine animals collected on November 3 kept in constant light (fluorescent lamp, 43 f. c.); temperature, 5°–8° C.

(9) Six animals collected on February 6 kept under aquarium conditions from February 7 to March 7, midday natural light throughout glass, 47 f. c.; temperature, 12°–17° C.

(10) Ten animals collected on February 6, temperature, 14.5°–17° C.; light increasing 15 minutes per day from 10 hours on February 7 to 16.5 hours on March 7 with an intensity of 18 f. c.

Figure 4 summarizes the results. Since normally during the winter months the inactive testes do not contain spermatocytes it seemed reasonable to suppose the presence of spermatocytes at this time was an indication of germinal activity. The animals collected on November 3 (group 1) and killed on February 7 did show some indications of germinal activity since 8.0 per cent of the testes were occupied by spermatocytes, while the animals collected on February 6 (group 2) had no spermatocytes and were in the typical winter condition. It is therefore clear that temperatures from 12°–17° C. were capable of initiating spermatogenesis at least to the extent of spermatocyte formation. As might be expected under these conditions the longer the animals were kept, the greater the number of spermatocytes were formed. Group 3, kept from November 3 to March 7, had this increase with 14.5 per cent spermatocytes. A striking increase to 33.8 per cent was found in the animals which had been subjected to increasing light (group 4). This might be interpreted as proof of a light effect, but the animals which had been kept in constant darkness had nearly as great an increase with 26.4 per cent spermatocytes, while animals under constant light had only 12.4 per cent

spermatocytes. As has already been pointed out there is considerable variation within groups; consequently, from these data it cannot be concluded that light has any effect. The low temperature groups (7

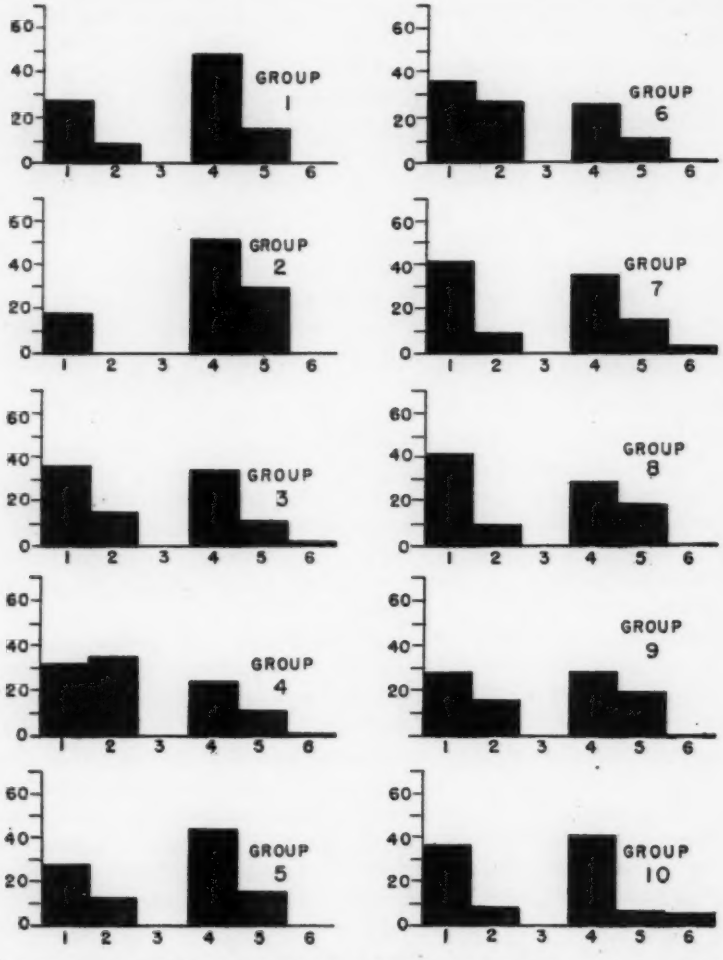


FIGURE 4. Results of Experiment 3 showing the average percentage of the testis occupied by the various germ cells. Refer to the text for the experimental conditions of the groups. Key: 1, Spermatogonia. 2, Spermatocytes. 3, Spermatids. 4, Sperm. 5, Evacuated cysts. 6, Non-germinal.

and 8) again tended to show that the germinal activity of the animals involved had been stopped as the spermatocyte percentages of 8.8 per cent and 9.7 per cent approximated the 8.0 per cent value of the animals killed at the beginning of the experiment (group 1). No degenerating cells were seen in the low temperature groups, possibly because of the shortness of the period of exposure. There were no significant light differences at the low temperatures.

The animals collected on February 6 also produced spermatocytes (compare groups 9 and 10 with group 2). The animals exposed to an artificial increase of light of 6.5 hours were not significantly different in spermatocyte production from those subjected to the natural daylight increase of 1.3 hours.

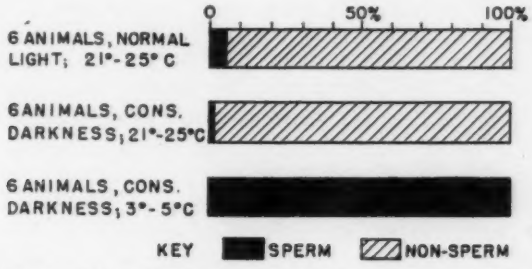


FIGURE 5. Results of Experiment 4 showing the percentage of the total weights of the testes occupied by sperm and non-sperm.

Statistical analysis (Fisher's method, 1938) showed no significant difference between the animals kept in constant darkness (group 6, 15°–17° C.) and those in the increasing light (group 4, 14°–16° C.) ($P = .4–.3$). Groups 9 and 10 were also not significantly different ($P = .6–.5$). Despite the fact that there was a significant difference between the increasing-light group (4) and the controls (group 3) ($P = .05–.02$) but not between the controls and the constant-darkness group ($P = .2–.1$) the total evidence does not point to increasing light as affecting spermatocyte production.

Experiment IV.—In order to determine the effects of light and temperature on the discharge of sperm, 18 animals were collected on October 28, 1940, and divided into three groups of six animals each. One group was placed under aquarium conditions with daylight through glass and room temperature 21°–25° C. The second was placed in constant darkness at 21°–25° C. A third was placed in constant darkness at 3°–5° C. The animals were killed on December 10 (period of 43 days), the testes weighed, separated into non-sperm and sperm regions,

and the percentage of the total testes' weight formed by each of these parts was determined. Figure 5 summarizes the results. Ordinarily at this season of the year (October 28–December 10) sperm are not discharged and the testes contain spermatogonia and a high percentage of sperm, but the animals at 21°–25° C. did discharge sperm to an approximately equal extent. The low-temperature (3°–5° C.) group showed the condition similar to that found in winter since their testes contained 99.41 per cent sperm. It is obvious from Figure 5 that light did not affect sperm discharge but temperature did.

Summary of Experimental Work

In Experiment I low temperatures halted spermatogenesis during summer, while on the other hand, room temperatures permitted the maturation processes to continue. Spermatocytes (probably secondary) and spermatids degenerated at the low temperatures. A similar low temperature effect was noted by Galgano (1936) in *Rana esculenta*. He found the germ cells increasingly sensitive to cold in the following order; primordial germ cells, spermatogonia, primary spermatocytes, secondary spermatocytes, and spermatids. *Triturus viridescens* did not show the same order of degeneration since spermatogonia and primordial germ cells were unaffected and the secondary spermatocytes were the most affected. Spermatids had pycnotic nuclei, but the secondary spermatocytes had completely degenerated thus leaving empty cysts. Since the cysts were empty it is not possible to state definitely that they were occupied by secondary spermatocytes but there is good evidence for this opinion since these cysts were located in the region between the primary spermatocytes and spermatids where secondary spermatocytes are usually found; furthermore, there were considerable amounts of normal primary spermatocytes present. It seems logical to assume that mitotic stages of the germ cells are more sensitive to cold than others since interference with mitosis often leads to abnormalities.

In Experiment II low temperatures were again seen to arrest germinal activity, this time at an earlier stage in the cycle. Degenerated cysts were not found, since spermatocytes and spermatids were absent at that time (May 2). In Experiment III winter animals were subjected to a temperature of approximately 15° C. At this temperature spermatocytes were formed but there was no measurable discharge of sperm, indicating, therefore, that different phases of the cycle require higher temperatures than others. At experimental temperatures of 21°–25° C. in winter, sperm were discharged and a new sperm cycle initiated (Experiment IV).

Light variations (constant light, increasing light, total darkness) did not alter spermatogenesis at any phase of the cycle at any of the temperatures used. A justifiable criticism of the light experiments, however, is that all of the intensities used may have been less than the threshold value or inadequate as to wave length; if this were true, differences in light rations could not reasonably have been expected to have been effective. In other forms, however, such as the starling, light intensities as low as 2.5 f. c. and of similar wave lengths were effective (Bissonnette, 1932) so that the 13 f. c. (lowest intensity used) and the wave lengths were probably entirely adequate. In all events the experiments show clearly that normal spermatogenesis can occur in the complete absence of light if the temperature is adequate. Therefore, it may be concluded with reasonable certainty that light is not an essential factor in the environmental control of the sperm cycle in Triturus.

Similarly lack of food did not inhibit spermatogenesis during the active season. While an extended study of this factor was not made, the evidence indicates that food supply plays no part, or at best a minor one, in the production of the normal rhythm.

Applying the evidence obtained from the experiments to the facts concerning the sperm cycle in nature, it is logical to conclude that temperature is the chief environmental factor influencing the sperm cycle. In fact it appears that temperature changes are responsible for the annual cycle in Triturus since the low temperatures of winter merely interrupt what would normally be a continuous spermatogenesis. Therefore, *Triturus viridescens* apparently does not possess an inherent annual sperm rhythm but has a yearly cycle imposed by environmental temperatures.

Discussion

The experimental evidence presented in this paper has led to the conclusion that temperature conditions are responsible for the annual sperm cycle in *Triturus viridescens*. This conclusion agrees with that of Galgano (1936) in his experiments with the frog, *Rana esculenta*. While he did not test light effects, he was able to induce spermtogenesis by increasing the temperature without additional illumination. In contrast to these results Bullough (1939) reports that Spaul and Gladwell (personal communication to him) found light was a requisite for the production of mature gametes and spawning in *Rana temporaria*.

The simplest explanation for the diversity between *R. temporaria* and *R. esculenta*, or any other group, may be that it is an actual one based on species differences. In fact, there may even be racial diversities as Witschi (1924) found in *R. temporaria*. There is, of course, good evi-

dence for species differences to be found in other classes. For example, Baker and Ranson (1938) list a number of species of southern hemisphere birds whose breeding cycles remain unchanged when brought to the northern hemisphere while the breeding cycles of others are modified by the new environment.

There are other possibilities to account for these variations and chief of these seems to be the lack of a uniform criterion for determining the environmental effects on the sexual cycle. The differences between *R. esculenta* and *R. temporaria* might be attributed to this cause, for the presence of sperm in the testes was Galgano's criterion of testicular activity while apparently Spaul and Gladwell used spawning as an indication of gonadal activity. Since this work is as yet unpublished, further discussion of it must be deferred. However, the possibilities that the sexes might respond differently to similar environmental changes should be considered. As an example, apparently there are sexual cyclic differences in *Triturus viridescens,* for during the "false" breeding season the males discharge sperm but the females do not ovulate (Pope, 1924). The cause of this disparity is not clear and is even more puzzling since, according to Adams (1940), the ovaries are fully mature at that time. This example indicates that sexual differences do exist and consequently in comparing results of different experiments it is not possible to use spawning as the criterion in one case and mature gametes of one of the sexes for the other.

Another source of confusion in the literature is the lack of quantitative methods for the measurement of the degree of gametogenesis. In experiments designed to indicate whether an environmntal factor, such as light, is ncessary for gametogenesis a quantitative study is not necessary providing the evidence is clear cut, as when one experimental group shows gametogenesis, and the other does not. The early experiments of Rowan (1925, 1926) were of this type since the gonads of the juncos subjected to increased light were activated while the controls were not. However, quantitative methods are necessary for valid results when various degrees of spermatogenesis are present but it is sometimes difficult to find a suitable method particularly with mammals and birds. This probably accounts for the many contradictory conclusions that have been reached in the experiments on these forms. The lack of experimental agreement makes it apparent that there is insufficient evidence upon which to base any general conclusions as to the environmental control of vertebrate breeding cycles. Thus Bullough suggests that marine fishes might be controlled by temperature because of the regularity of that factor in the sea, while fresh-water forms might find temperature erratic in shallow bodies of water and hence would find the regular fluc-

tuation of light more advantageous. Whether such control would be more advantageous to fresh water forms is difficult to determine. At any event, the sperm cycle of *Triturus viridescens*, a fresh water amphibian, apparently is limited by the temperatures of the environment.

Summary

1. The observations of Adams (1940) on the normal sperm cycle of *Triturus viridescens* were confirmed. An active period of gametogenesis following the spring breeding season results in the formation of mature sperm by September. Mature sperm are discharged in the fall but during winter the testes are inactive, consisting of spermatogonia, sperm, and evacuated cysts. Sperm discharge recurs in the spring.

2. At all periods of the cycle temperatures below 12° C. prevented spermatogenesis and sperm discharge and caused the degeneration of spermatocytes and spermatids. Temperatures above 12° C. induced spermatocyte formation in winter; 21°–25° C. in winter caused sperm discharge and in summer these temperatures allowed normal spermatogenesis.

3. Variations in light rations (constant light, increasing light, constant darkness) did not affect the cycle. Lack of food did not inhibit spermatogenesis.

4. It is concluded that temperature is the principal environmental agent influencing the sperm cycle of *Triturus viridescens*.

LITERATURE CITED

Adams, A. E., 1940. Sexual conditions in Triturus viridescens. III. The reproductive cycle of the adult aquatic form of both sexes. *Am. Jour. Anat.*, **66**: 235–275.

Alexander, S. S., and C. W. Bellerby, 1935. The effect of captivity upon the reproductive cycle of the South African clawed toad (Xenopus laevis). *Jour. Exp. Biol.*, **12**: 306–314.

Alexander, S. S., and C. W. Bellerby, 1938. Experimental studies on the sexual cycle of the South African clawed toad (Xenopus laevis). I. *Jour. Exp. Biol.*, **15**: 74–81.

Baker, J. R., and R. M. Ranson, 1938. The breeding season of southern hemisphere birds in the northern hemisphere. *Proc. Zool. Soc. Lond.*, A, **108**: 101–141.

Bellerby, C. W., 1938. Experimental studies on the sexual cycle of the South African clawed toad (Xenopus laevis). II. *Jour. Exp. Biol.*, **15**: 82–90.

Bellerby, C. W., and L. Hogben, 1938. Experimental studies on the sexual cycle of the South African clawed toad (Xenopus laevis). III. *Jour. Exp. Biol.*, **15**: 91–100.

Bissonnette, T. H., 1932. Studies on the sexual cycles in birds. VI. Effects of white, green, and red lights of equal luminous intensity on the testis activity of the European starling (Sturnus vulgaris). *Physiol. Zool.*, **5**: 92–123.

Bissonnette, T. H., 1936. Sexual photoperiodicity. *Quart. Rev. Biol.*, **11**: 371–386.

BISSONNETTE, T. H., 1937. Photoperiodicity in birds. *Wilson Bull.*, **49**: 241–270.

BLES, E. J., 1905. The life-history of Xenopus laevis, Daud. *Trans. Roy. Soc. Edin.*, **41**: 789–821.

BULLOUGH, W. S., 1939. A study of the reproductive cycle of the minnow in relation to the environment. *Proc. Zool. Soc. Lond.*, A, **109**: 79–102.

FISHER, R. A., 1938. Statistical methods for research workers. 7th edition. Edinburgh and London.

GALGANO, M., 1931. Osservazioni e considerazioni intorno ai processi spermatogenetici normale ed aberranti di Rana esculenta L. *Monit. Zool. Ital.*, **42**: 297–307.

GALGANO, M., 1932. Prime richerche intorno all'influenza della temperatura sui processi spermatogenetici normale ed aberranti di Rana esculenta L. *Monit. Zool. Ital.*, **43**: 156–160.

GALGANO, M., 1934. L'influenza della temperatura sulla spermatogenesi della Rana esculenta L. *Monit. Zool. Ital. Supp.*, **45**: 83.

GALGANO, M., 1936. Intorno all'influenza del clima sulla spermatogenesi di Rana esculenta L. *Arch. Ital. Anat. e Embriol.*, **35**: 511–541.

HILSMAN, H. M., 1932a. Germ-cell cycles in the amphibian Triturus viridescens. *Anat. Rec.*, **54**, supp. p. 107.

HILSMAN, H. M., 1932b. Seasonal changes in the gonads of the amphibian, Triturus viridescens. *Proc. Pa. Acad. Sci.*, **6**: 1–3.

HOGBEN, L. T., E. CHARLES, AND D. SLOME, 1931. Studies on the pituitary. VIII. The relation of the pituitary gland to calcium metabolism and ovarian function in Xenopus. *Jour. Exp. Biol.*, **8**: 345–354.

MARSHALL, F. H. A., 1936. Sexual periodicity and the causes which determine it. *Phil. Trans. Roy. Soc. Lond.*, B, **226**: 423–456.

POPE, P. H., 1924. The life of the common water-newt (Notophthalmus viridescens), together with observations on the sense of smell. *Ann. Carnegie Mus.*, **15**: 305–368.

ROWAN, W., 1925. Relation of light to bird migration and developmental changes. *Nature, Lond.*, **115**: 494–495.

ROWAN, W., 1926. On photoperiodism, reproductive periodicity, and the annual migration of birds and certain fishes. *Proc. Boston Soc. Nat. Hist.*, **38**: 147–189.

ROWAN, W., 1938a. London starlings and seasonal reproduction in birds. *Proc. Zool. Soc. Lond.*, A, **108**: 51–77.

ROWAN, W., 1938b. Light and seasonal reproduction in animals. *Biol. Rev.*, **13**: 374–402.

SHAPIRO, H. A., 1936. The biological basis of sexual behavior in amphibia. I. The experimental induction of the mating reflex (coupling) in Xenopus laevis (the South African clawed toad) by means of pregnancy urine and of anterior pituitary extracts, with the production of fertilized ova. *Jour. Exp. Biol.*, **13**: 48–56.

SHAPIRO, H. A., 1937. The biological basis of sexual behaviour in amphibia. IV. *Jour. Exp. Biol.*, **14**: 38–47.

SHAPIRO, H. A., AND H. ZWARENSTEIN, 1933. Metabolic changes associated with endocrine activity and the reproductive cycle in Xenopus laevis. I. The effects of gonadectomy and hypophysectomy on the calcium content of the serum. *Jour. Exp. Biol.*, **10**: 186–195.

WITSCHI, E., 1924. Die Entwicklung der Keimzellen der Rana temporaria L. I. Urkeimzellen und Spermatogenese. *Zeitsch. f. Zell. u. Geweb.*, B, **1**: 523–561.

ZINN, D. J., AND J. D. IFFT, 1941. A new limnophotometer with a special adaptation for the measurement of the penetration of light through ice under natural conditions. *Ecology*, **22**: 209–211.

THE AUTOLYSIS OF MUSCLE OF HIGHLY ACTIVE AND LESS ACTIVE FISH

BASIL BAILEY, P. KORAN, AND H. C. BRADLEY

(From the Laboratories of Physiological Chemistry, University of Wisconsin, Madison, and the Marine Biological Laboratory, Woods Hole

Most of the studies of autolysis have been made upon such mammalian tissues as liver, spleen, kidney and muscle. Sufficient data have also accumulated to indicate that all vertebrate tissues behave essentially like mammalian tissues and may be presumed therefore to contain the same enzyme complex responsible for the autolysis and atrophy of mammalian tissues. Primary cleavage is accomplished by cathepsin—which may be a complex rather than a single proteinase—with an optimum at about pH 4. At pH 2 there is no digestion and the enzyme is destroyed rapidly; at pH 7 digestion is zero or nearly so. At pH 4 to 6 the enzyme may survive several weeks (Eder, Bradley, and Belfer, 1939), so that cessation of digestion within this pH range and period of time cannot be attributed to loss of enzyme activity, but to lack of substrate.

In some preliminary studies of fish muscle (Chen and Bradley, 1924) there was evidence of a correlation between the activity of the organism as a whole, and the speed and extent to which autolysis would proceed. These data were secured before accurate pH control could be assured, and by methods which gave total cleavage rather than primary proteolysis. The measurements therefore were an expression of the composite activity of proteinase together with the various peptidases present in the tissues.

It seems desirable therefore to reinvestigate this phenomenon with the more adequate technique now available, and to extend the observations.

The material was obtained for the most part alive. In two instances fish were frozen and shipped under refrigeration to the laboratory at Madison from the Pacific Coast. It has been shown that the autolysis of such material is essentially like that of freshly killed tissues (Callow, 1925). In addition to the highly active group and the rather sluggish group reported in detail here, we have examined a number of others of intermediate activity, such as the mackerel shark, sand shark, electric ray, flounder, scup and haddock. The muscles of these fish autolyze with the same characteristic pattern and to an intermediate extent. Inasmuch as we have no reliable data on the degree of activity, rate of

metabolism, speed, strength and endurance of any fish, the material we present here was secured from two groups differing very obviously in speed and activity. The rather sluggish or moderately active group comprises the carp (*Cyprinius carpio*), and gar (*Lepisosteus osseus*) from the Wisconsin streams and lakes, and the cod (*Gadus callarias*) and dogfish (*Squalus acanthias*) taken from the Atlantic ocean near Woods Hole, Massachusetts. A second group picked for its outstanding activity, speed and gamey qualities consisted of the Pacific mackerel (*Scomber scombrus*), albacore (*Germo alalunga*); the bonita (*Sardia sarda*), mackerel (*Scomber scombrus*) and swordfish (*Xiphias gladius*) from the Atlantic.

Experimental Procedure

The fish muscle dissected from several specimens to give a representative composite value was ground fine and weighed out to make a 20 per cent mixture in water, preserved with 5 per cent toluol. The material was homogenized in a Waring Mixer to the consistency of a smooth cream, taking care to avoid the inclusion of air bubbles. One hundred cc. digests were made from this stock, and the pH of each digest adjusted to the desired level by means of 5N HCl or N NaOH, using the glass electrode. Samples were removed at once and from time to time later; diluted with one volume of water and precipitated with two volumes of 10 per cent trichloracetic acid. At this concentration none of the native proteins appear in the filtrate, but the fragments from early cleavages do. Digestion was determined by increase in the soluble nitrogen and by the increase of the tyrosine reaction of Folin and Ciocalteu (1927) measured either by colorimeter or with the Photelometer. Nitrogen was determined by the macro-Kjeldahl method when available, and by the micro-Kjeldahl technique of Folin when not. The latter is less accurate but all results represent duplicate analyses. These two methods under the conditions used give independent evidence of the early cleavage stages of the tissue proteins and very closely parallel each other. The pH of each digest must be readjusted frequently during the first three days, since it shifts rapidly as digestion proceeds. After three days it remains substantially constant indefinitely. All digests were maintained at 38° C. Identical series were carried out to which 3 cc. of 20 per cent hemoglobin, purified as described by Anson and Mirsky (1933), were added as additional available substrate.

Each digestion series was prepared from the muscles of several specimens. The results are therefore composite and probably represent the average behavior of the species better than the muscles of a single specimen. Many of the experiments were repeated during the following year

to check previous findings. While there are differences between single specimens and groups of specimens obtained at different times, the essential behavior is reproducible. Thus carp muscle, tested many times, always autolyzes slowly and to a small extent; mackerel rapidly and more extensively.

We present a typical protocol obtained with muscle of the gar, in Table I. All of the series were carried out in the same way and under identical conditions.

TABLE I

Muscle Gar No. 1

	Muscle 20 gm./100 cc.	pH	Mg. Tyrosine/1 cc. filtrate					Mg. N_2/1 cc. filtrate				
			Initial	Increase in Tyrosine				Initial	Increase in sol. N_2			
				Days					Days			
			0	1	3	5	10	0	1	3	5	10
1	Control	6.4	.009	.003	.006	.008	.017	.134	.007	.028	.028	.039
2	"	6.0	"	.006	.014	.019	.033	.144	.005	—	.033	.039
3	"	5.0	"	.016	.030	.038	.057	.140	.043	.073	.113	.130
4	"	4.4	"	.026	.041	.049	.071	.133	.066	.133	.186	.229
5	"	3.6	"	.052	.073	.080	.102	.122	.173	.277	.357	.423
6	"	2.6	"	.032	.052	.057	.083	.128	.088	.119	.204	.298
7	"	1.7	"	.005	.007	.007	.012	.134	.025	.052	.078	.122
8	"	1.0	"	.002	.005	.008	.012	.133	.024	.081	.080	.113
9	"	7.5	"	.002	.004	.005	.007	.144	.006	.006	.009	.022
10	Control+3 cc. 20% hemoglobin	6.5	.010	.004	.009	.013	.019	.133	.020	.040	.061	.067
11	"	6.2	"	.009	.021	.027	.038	.144	.022	.029	.069	.103
12	"	5.4	"	.032	.058	.070	.101	.136	.090	.172	.223	.239
13	"	5.0	"	.052	.084	.096	.121	.146	.186	.290	.332	.365
14	"	4.0	"	.093	.148	.174	.206	.136	.321	.616	.730	.795
15	"	3.4	"	.089	.145	.164	.191	.146	.396	.584	.665	.726
16	"	2.0	"	.017	.023	.028	.031	.146	.047	.054	.079	.116
17	"	1.3	"	.003	.007	.009	.015	.144	.003	.042	.069	.122
18	"	7.5	"	.004	.007	.009	.011	.146	.012	.016	.020	.080

In presenting the rest of our experimental data we shall use the graphic method, and are omitting the three- and five-day digestion curves for the sake of simplicity. Digestion in one day represents essentially the *speed* of the reaction, since there still remains a large excess of substrate. Ten-day digestion approximates final equilibrium and represents therefore the *extent* of autolysis.

In Figure I is shown the autolytic pattern of carp muscle, which represents very well the other members of the less active group. In the

ten-day period about 30 per cent of the total muscle protein had digested. Added hemoglobin is nearly completely digested in the time allotted to the experiment. Digestion was still in progress at the end of ten days, and somewhat more of the muscle proteins would have undergone cleavage if the time had been extended.

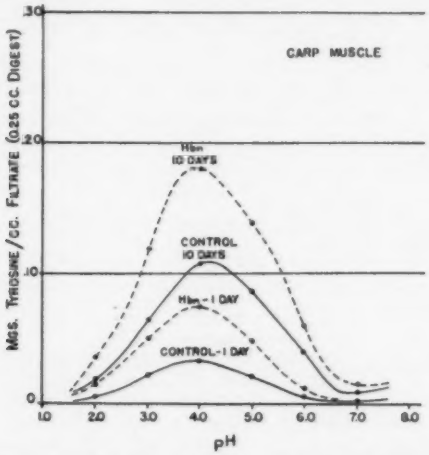


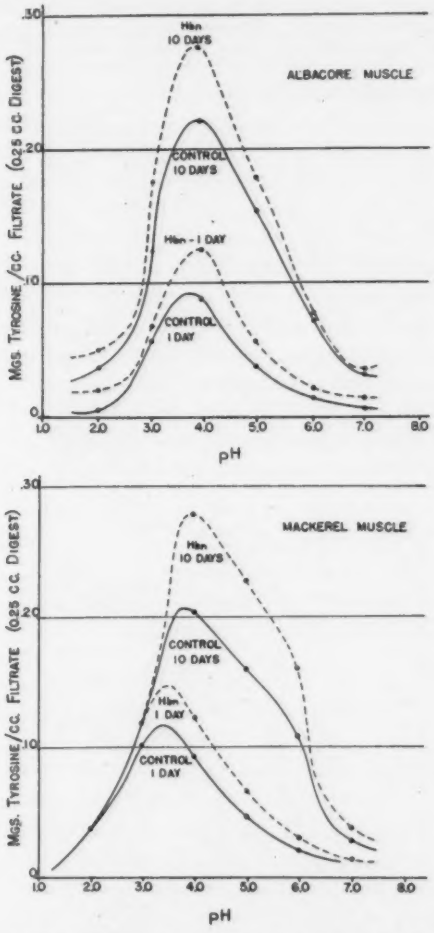
FIGURE I. The curves indicate cleavage as measured by the tyrosine color, attained by a series of digests in 24 hours where pH levels were set and maintained. Three- and five-day curves are omitted. Tissue alone is represented by solid lines; tissue and hemoglobin by dotted lines. The vertical distance between the two curves of the same duration represents the amount of tyrosine referable to the added substrate hemoglobin. In this muscle it is evident that half of the total tyrosine liberated in 24 hours came from hemoglobin cleavage.

Autolysis of carp muscle may be compared with that of albacore and Pacific mackerel under identical conditions, Figures II and III.

It will be noted that relatively more hemoglobin digests the first day in carp muscle autolysis than in the autolysis of mackerel or albacore muscle. On the other hand there is much more of the active mackerel and albacore muscle protein digested in one day than in the case of the carp. This difference is summarized in Table II.

Assuming that the tissue proteinase distributes itself between the muscle proteins and the hemoglobin in proportion to the relative masses of the two proteins, then it would appear that carp muscle is less easily fragmented than hemoglobin, while mackerel and albacore proteins are more easily fragmented by the tissue proteinase. The data suggest that the muscle proteins of the active fish are more fragile to the enzymes

present than are the proteins of the less active fish muscles. Whether there is more enzyme present in the more active muscles than in the less active is not clear from our data and will require the development of



FIGURES II and III. The rapid autolysis of these muscles is evident from the curves. Available muscle proteins are abundant so that added substrate does not greatly increase tyrosine liberated in 24 hours. By the tenth day the increased tyrosine from hemoglobin represents the nearly complete cleavage of the latter. It will be noted that digestion proceeds more slowly from five to six but in ten days is very considerable.

TABLE II

Subtracting the tyrosine produced by fish muscle alone from that produced from muscle plus hemoglobin, gives the amount referable to cleavage of hemoglobin. It will be seen that while the total tyrosine is larger in the game fish muscle digests in one day, the proportion derived from hemoglobin in the latter is considerably less, and is actually somewhat less also. This we believe indicates a preferential splitting of muscle proteins in the case of the game fish group, which indicates their greater fragility.

	Mg. Tyrosine in 1 day from			Per cent Tyrosine in 1 day from	
	Muscle	Hemoglobin	Total	Muscle	Hemoglobin
Carp	0.33	0.45	0.75	44	56
Albacore	0.95	0.32	1.27	75	25
Mackeral	1.17	0.30	1.47	80	20

adequate technique for extracting all of the enzyme present in the tissue for comparison when using a standard substrate like hemoglobin. We expect to investigate this point further.

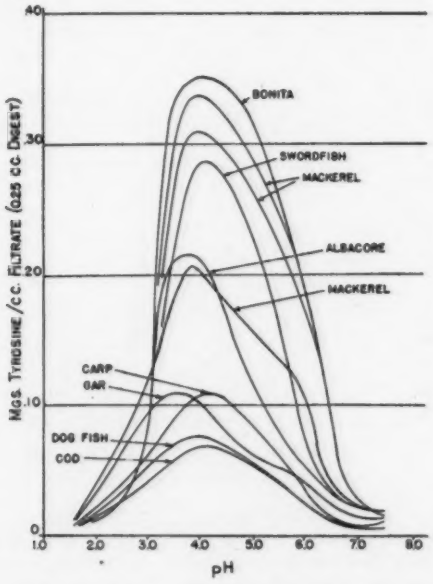


FIGURE IV. The curves above illustrate the contrast in protein cleavage between the active and less active group. Digests are of muscle only.

In Figure IV we have grouped the results of typical autolyses carried on for ten days in which the behavior of the muscles of the sluggish types is contrasted with that of the highly active. No hemoglobin was present in these digests, which indicate the extent of tissue cleavage accomplished in the ten-day period in the two groups. In the active group the cleavage level represents substantially the final equilibria attainable. In the less active forms, where digestion is slower, cleavage has not quite reached final equilibrium.

The difference in autolysis between the two groups emphasizes again the correlation between the extent and speed of digestion attained in ten days and the normal functional activity of the muscles. Pending further studies we believe the rapid autolysis of the game fish muscle proteins represents an ability to mobilize tissue proteins rapidly for the maintenance of high level activity, when the usual stores of glycogen and fat have been depleted. Greene (1919) has shown that in its fasting migration to the spawning beds the king salmon mobilizes 30 per cent of its muscle proteins without sacrifice of essential contractile structures. We assume that in the rapid autolysis of the muscles of game fish here described we have the machinery for similar mobilization of stored protein for fuel requirements when emergencies develop for these rapidly metabolizing species.

Summary

1. All fish muscles examined show the typical pattern of catheptic activity characteristic of such mammalian tissues as liver, kidney, spleen and muscle.

2. Muscles of the relatively sluggish fish such as carp, gar, dogfish and cod autolyze slowly and to a relatively small extent in the ten-day period of the experiments.

3. Muscle from the game fish mackerel, albacore, bonita and swordfish autolyze much more rapidly and completely in the same time, and under the same conditions.

4. A correlation appears to exist between the normal functional activity of the muscles and the speed and extent to which they digest. This represents we believe a mechanism for maintenance of activity through mobilization of tissue proteins during periods of food scarcity.

5. The data suggest that muscle proteins of the active species are more readily fragmented by the enzymes present than are the proteins of less active species.

LITERATURE CITED

ANSON, M. L., AND MIRSKY, A. E., 1933. The estimation of trypsin with hemoglobin. *Jour. Gen. Physiol.*, **17**: 151–157.

CHEN, K. K., AND BRADLEY, H. C., 1924. The autolysis of muscle. *Jour. Biol. Chem.*, **59**: 151–164.

CALLOW, E. H., 1925. The autolysis of the muscle of the codfish. *Jour. Biochem.*, **19**: 1–6.

EDER, H., BRADLEY, H. C., AND BELFER, S., 1939. The survival of cathepsin in autolysis. *Jour. Biol. Chem.*, **128**: 551–557.

FOLIN, O., AND CIOCALTEU, V., 1927. On tyrosine and tryptophane determination in proteins. *Jour. Biol. Chem.*, **73**: 627–650.

GREENE, C. W., 1919. Biochemical changes in the muscle tissue of king salmon during the fast of spawning migration. *Jour. Biol. Chem.*, **39**: 435–456.

TRITURUS TOXIN: CHEMICAL NATURE AND EFFECTS ON TISSUE RESPIRATION AND GLYCOLYSIS

W. J. VAN WAGTENDONK,[1] F. A. FUHRMAN, E. L. TATUM AND J. FIELD, II [2]

(Schools of Biology and of Medicine, Stanford University, California)

Introduction

The eggs and embryos of certain Triturus salamanders contain a toxic principle which selectively paralyzes the motor portion of the nervous system of Amblystoma salamander larvae (Twitty, 1937). This toxin, which seems to be neither an alkaloid nor a substance related to the cardiac toad poisons, caused death in mammals (usually preceded by convulsions) by direct depression of the central respiratory mechanism (Horsburgh, Tatum and Hall, 1940). Further investigations into the chemical nature and the mode of physiological action of the toxin are reported in this paper.

Chemical Section

While the toxin has not yet been identified in a chemical sense, it has been prepared in more concentrated solutions than were hitherto available. It is now possible to rule out the possibility of identity with a number of biologically active agents. The evidence on this point follows.

Materials and Methods

Eggs of *Triturus torosus* were crushed, pressed, and the juice filtered through cloth. Ethyl alcohol was added to the filtrate to a concentration of 50 per cent, so that the solution could be stored until needed. In all, 24 liters of this solution were obtained from seven or eight gallons of egg clusters, with a total activity of 600,000 *mouse units*.

The toxin content of all preparations was assayed biologically as previously described (Horsburgh, Tatum and Hall, 1940) and calculated in terms of the *"mouse unit"* (m.u.). One *m.u.* is that amount which on subcutaneous injection completely stops the respiration of a white mouse in 10 minutes.

[1] Now at Oregon State College, Corvallis, Oregon.

[2] Supported in part by grants from the Stanford University School of Medicine and from the Rockefeller Foundation.

Preliminary investigations. The stability of toxin solutions to heating and to acid and alkali was found to be essentially as previously given (Horsburgh, Tatum and Hall, 1940). It was stable at 100° C. at pH 6 or 7, was 80 per cent inactivated in 30 minutes at pH 4, and 60 per cent inactivated at pH 8 and pH 10. At pH 9 and 120° C. the toxin was over 99 per cent inactivated in 10 minutes. Inactivated toxin solutions were prepared in this way for use in the respiratory studies. It was completely destroyed at pH 12 even at room temperature in a few minutes.

Although the solubilities of the toxin were essentially as previously reported (Horsburgh, Tatum and Hall, 1940), the present material appeared to be much more unstable in higher concentrations of alcohol. It was partially or largely inactivated in concentrations of ethyl alcohol and acetone over 75 per cent, and in methyl alcohol over 90 per cent. However, in the presence of NaCl, it is quite stable, even in absolute methyl alcohol, in which it is completely soluble. This stabilizing effect of salt is evident even in water solutions of the toxin, which gradually decrease in activity on standing unless around 1 per cent NaCl is present. No explanation of this salt effect is available at present.

The chemical evidence substantiates the earlier conclusion that the toxin is not basic in nature. It was not precipitated from concentrated solutions by any basic precipitants tried, including chlorplatinic, phosphotungstic, picric, picrolonic, and flavianic acids.

Attempts were made to purify the material by selective adsorption of the toxin on activated charcoal and on kaolin. Both of these either adsorbed or inactivated the toxin, but no active material could be recovered from the adsorbent, although a number of solvents (pyridine, alcohol and acetone), at several pH values, were tried.

High vacuum molecular distillation of the toxic principle was also unsuccessful. No active material distilled at 10^{-5} mm. Hg at temperatures of 80° C. or higher. After heating to 140° C. the residue was also inactive.

An estimation of the molecular weight by measuring the rate of diffusion through agar blocks (Tatum and Beadle, 1938) and testing the concentration of toxin in each block by the mouse test, gave results indicating a molecular weight between 200 and 400. This value and the ease with which the toxin passes through a dialyzing membrane rule out high-molecular compounds such as proteins or peptones. (See also evidence of rapid diffusion in embryos, Twitty, 1937.)

Final purification procedure. In spite of the rather great instability of the toxin, it has been possible, by making use of the most successful and

least destructive treatments, to purify the active principle to a considerable extent. It was found necessary to work up only small batches at a time, since otherwise too much activity was lost. The most satisfactory procedure was essentially as follows:

Preparation I: 3 liters toxin solution in 50 per cent ethyl alcohol (14.4 gm. dry weight; 72,000 *m.u.*). Activity: 5,000 *m.u.* per gm.; treated with super cel; filtered.

Preparation II: Filtrate (60,000 *m.u.*). Concentrated in vacuo to 75 cc.; dialyzed against 1 per cent NaCl solution.

Preparation III: Dialyzate (60,000 *m.u.*). Concentrated to dryness in vacuo; extracted with hot absolute methyl alcohol.

Preparation IV: Extract (54,000 *m.u.*). Dried, extracted with hot chloroform.

Preparation V: Residue (1.61 gm. dry weight; 50,000 *m.u.*). Activity: 31,000 *m.u.* per gm.; dissolved in 5 cc. 1 per cent NaCl solution; phosphotungstic acid in 1 per cent NaCl solution added until precipitation was almost complete; centrifuged.

Preparation VI: Centrifugate (0.66 gm. dry weight;[3] 42,500 *m.u.*). Activity: 64,400 *m.u.* per gm.

It was found that the recovery of the toxin in the last step was most satisfactory if an excess of phosphotungstic acid was avoided. Although the toxin was not precipitated by an excess of phosphotungstic acid, even in the presence of sulfuric acid, the resulting preparation lost its activity very much more rapidly. This was true even in a 1 per cent NaCl solution, in which the toxin as ordinarily prepared was quite stable. It is possible that a precipitation with slight excess of phosphotungstic acid removed some protecting substances from solution.

The effect of various treatments on this purified preparation was investigated. It was easily decomposed by alkali, but without any detectable evolution of amines. The activity was unaltered by treatment with dilute bromine water, with Chloramine T, with formaldehyde, and by acetylation with acetic anhydride in methyl alcohol. The stability of the toxic principle to these reagents indicates that it probably does not contain an aliphatic amino group, and suggests that the amino acids present in this fraction are inactive contaminants.

Analyses of the most purified preparation. The best toxin preparation (VI) was analyzed for total-N (Pregl, 1935), and amino-N (Van Slyke, 1912) before and after hydrolysis at 120° C. with 10 per cent sulfuric acid, for ammonia-N and for reducing sugars (Stiles, Peterson and Fred, 1926). The results are given in Table I and are calculated on the basis of total organic material present in the preparation.

The results of the analyses show that 10 per cent of the organic dry material in this preparation was sugar, which presumably was inactive.

[3] Organic material.

No glycosidic sugars could be detected. No peptide-N was detected, and practically all of the total-N was accounted for by the amino- and ammonia-N. Although acetylation of the amino nitrogen was almost complete, there was no accompanying change in activity. This fact, together with the stability of the toxin to treatment with bromine water, chloramine-T, and to formaldehyde, suggests that the toxin itself contains no ordinary aliphatic amino-N. It may be significant that the ammonia-N, the non-acetylated amino-N and the amino-N lost on acid hydrolysis are of nearly the same magnitude.

TABLE I

Analysis of Toxin Preparation VI

Constituent	Per cent
Reducing sugars as glucose	10.9
Total-N	6.4
Ammonia-N	0.3
Amino-N	5.8
Amino-N after hydrolysis [1]	5.5
Amino-N after acetylation [2]	0.4

[1] Hydrolyzed with 10 per cent H_2SO_4, 3 hours at 120° C.

[2] Treated with acetic anhydride in absolute methyl alcohol solution; reagents removed in vacuo.

The activity of the preparation used for the analyses was 64 *m.u.* per milligram, which compares quite well with the previously reported activity of 75 *m.u.* per milligram (Horsburgh, Tatum and Hall, 1940). In order to estimate the potency of the pure toxin itself, the activities of various fractions of the preparation have been calculated from the analytical data given in Table I. For purposes of the calculations the amino acid content was taken as the amino-nitrogen value $\times$ 6.25. The activity of the material with the amino-acids and sugar removed, would then be around 120 *m.u.* per mg. However, it seems probable by analogy with other physiologically active materials that the toxin does contain nitrogen. The concentration of active material was therefore calculated on the basis of the non-acetylated amino-N, assuming a molecular weight of 400 for the toxin (the maximum value from the diffusion experiments) and 2 N atoms per molecule. The value obtained approximates 2,000 *m.u.* per mg. Similar values are obtained on the basis of ammonia-N or amino-N lost on acid hydrolysis. Even with half of this activity (1,000 *m.u.* per mg.), the material would still be extremely active as compared with other known toxic substances.

Physiological Section

The influence of Triturus toxin, prepared as described above, on respiration (preparation VI) and anaerobic glycolysis (preparation V) in isolated rat cerebral cortex and on respiration in isolated rat kidney cortex and liver is reported here.[4]

Materials and Methods

Fourteen adult albino rats were used. These were decapitated, the brains removed rapidly and placed in a moist box to minimize changes in water content during slicing (Sperry and Brand, 1941). Methods of slicing (Crismon and Field, 1940) and of preparation of toxin solutions for use in respirometer vessels (Fuhrman and Field, 1941) have been described elsewhere. Oxygen consumption and anaerobic glycolysis were measured by the usual manometric methods (Dixon, 1934) and the gas consumption or production was calculated in μ l., N.P.T., per mgm. wet weight per hour (Q_{O_2} and $Q_{N_2}^{CO_2}$ respectively). The thermostat temperature was $37.5° \pm 0.01°$ C. Toxin solutions to be added were adjusted to the pH of the suspension medium, and frequent checks with a glass electrode showed that little change in pH of the suspension medium occurred during a run.

Toxin was first added to the experimental vessels from the sidearm after a preliminary run of 30 minutes. At least two vessels were run at each concentration (active or inactivated) and, as controls, two vessels receiving no toxin. Frequent checks (Horsburgh, Tatum and Hall, 1940) showed that the toxin did not decrease in potency during the runs.

Results

1. *Respiration of Brain, Kidney and Liver.*—It is shown in Table II, part 1, that differences in Q_{O_2} between the control and experimental runs were rather small and transient, and that the Q_{O_2} of cerebral cortex and kidney slices was affected only by the most concentrated toxin preparation available (850 *m.u.* per ml.), while the respiration of liver slices was stimulated slightly at a toxin concentration of 85 *m.u.* per ml. However, it is not clear that these differences in respiration could be attributed to the toxin, since concentrations of 85 and 850 *m.u.* per ml. involved the presence of 1.33 and 13.3 mgm. of organic matter respectively. These substances may have served as additional substrate.

Since addition of partially inactivated toxin gave results unlike those obtained with untreated dilute toxin of the same potency and since these

[4] A preliminary note dealing with the effects of toxin preparations on tissue oxygen consumption has been published (Fuhrman and Field, 1941).

results were unlike those observed in controls, it appears probable that inactivation involved more complex changes than simple destruction of toxin. Accordingly the partially inactivated toxin cannot be used to test the possibility that substrate rather than toxin was responsible for the questionable initial rise in Q_{O_2} at high toxin concentrations.

TABLE II

Part 1

Comparison of respiratory rates of rat cerebral cortex, kidney cortex and liver slices in the presence of high concentrations of Triturus toxin with that of controls (arbitrarily taken as 100). Time indicated is after start of run.

	Toxin				"Inactivated toxin"	
	850 *m.u.* per ml.		85 *m.u.* per ml.			
	30 min.	120 min.	30 min.	120 min.	30 min.	120 min.
Cerebral cortex	111	104	99	96	118	115
Kidney cortex	134	107	100	97	108	84
Liver	174	113	119	128	108	60

[1] Residual potency 85 *m.u.* per ml.

TABLE II

Part 2

Comparison of rates of anaerobic glycolysis in rat cerebral cortex in the presence of high concentration of toxin with that of controls (arbitrarily taken as 100)

	Toxin (*m.u.* per ml.)		Inactivated toxin [1] (initial *m.u.* per ml.)	
	37	112	37	112
Average rate during first 10 minutes after addition of toxin	253	344	290	250
Average rate for period 10 to 70 minutes after addition of toxin	135	36	167	70

[1] Residual potency less than 0.5 *m.u.* per ml.

2. *Anaerobic Glycolysis in Cerebral Cortex.*—The results of addition of toxin, in final concentrations of 37 and 112 *m.u.* per ml., are shown in Table II, part 2. There is a transient initial increase in the rate of carbon dioxide displacement which is probably due to some factor other than the toxin, since it was obtained with the inactivated as well as the active preparation. During the subsequent 60 minutes the higher concentration of toxin clearly inhibited anaerobic glycolysis. The

effect of the lower concentration was inhibitory also, since in its presence the rate of carbon dioxide production was somewhat lower than in the case of the "inactivated" control.

Discussion

Administration of Triturus toxin to the anesthetized cat in doses of 50 *m.u.* or more per kgm. body weight, caused death in about one minute, due to direct depression of the central respiratory mechanism (Horsburgh, Tatum and Hall, 1940). The concomitant fall in arterial pressure was insufficient to account for this effect on the basis of an interruption in the oxygen supply to the brain (Hall). The time involved appears too short for the development of fatal hypoglycemia, since we have found that the respiration of isolated cerebral cortex, in glucose-free media, does not diminish markedly in the first few minutes of measurement (cf. also MacLeod, 1934). However, the general picture, that of convulsions followed by rapid respiratory death, did suggest interference with the metabolism of the brain (cf. Gerard, 1938), possibly by inhibition of oxygen consumption, glycolysis or both, through action upon enzymes concerned in these processes. This seemed the more probable because certain snake venoms are known to inhibit one or both of these processes (Chain and Goldsworthy, 1938; Chain, 1937, 1939; Mellanby, 1935). However, under the conditions of our experiments, concentrations causing rapid respiratory death in intact animals have no effect on cerebral cortex respiration or anaerobic glycolysis (assuming uniform distribution). Very high concentrations of the toxin caused slight transient stimulation of respiration and inhibition of glycolysis. These were respectively, 17,000 and 7,000 times the concentration killing a cat in one minute. It thus appears that another explanation of the action of the toxin must be sought. It is possible that the toxin inhibits those reactions which underlie neuron excitation (conduction and transmission) in contrast to those important in resting maintenance or recovery.

Summary

The most active toxin preparation obtained had a potency of 64,000 *mouse units* (*m.u.*) per gram of organic material. Most of the nitrogen in this preparation was probably contained in inactive amino-acids, and the activity as calculated on the basis of the remaining nitrogenous material was between 1 and 2 million *m.u.* per gram.

The effect of the toxin on respiration and glycolysis in certain organs of the rat was investigated. Oxygen consumption of liver slices ap-

peared to be slightly and temporarily increased in the presence of 85 *m.u.* of toxin per ml. Similar effects were observed on the respiration of kidney and brain slices with a toxin concentration of 850 *m.u.* per ml. However, control studies with inactivated toxin makes it questionable whether these changes were attributable to the toxin *per se*.

Anaerobic glycolysis in rat brain slices was progressively inhibited with higher concentrations of the toxin preparation. However, this effect was obtained only with concentrations far higher than those required to kill a two-kilogram cat in one minute.

LITERATURE CITED

CHAIN, E., 1937. Effect of snake venoms on glycolysis and fermentation in cell-free extracts. *Quart. Jour. Exp. Physiol.*, **26**: 299–303.

CHAIN, E., 1939. Inhibition of dehydrogenases by snake venom. *Jour. Biochem.*, **32**: 407–411.

CHAIN, E., AND L. J. GOLDSWORTHY, 1938. Studies on the chemical nature of the anti fermenting principle in black tiger snake-venom. *Quart. Jour. Exp. Physiol.*, **27**: 375–379.

CRISMON, J. M., AND J. FIELD, 2ND, 1940. The oxygen consumption in vitro of brain cortex, kidney, and skeletal muscle from adrenalectomized rats. *Amer. Jour. Physiol.*, **130**: 231–238.

DIXON, M., 1934. Manometric methods. Cambridge University Press, London.

FUHRMAN, F. A., AND J. FIELD, 2ND, 1941. Effect of Triturus toxin on respiration of rat tissues in vitro. *Proc. Soc. Exp. Biol. and Med.*, **48**: 423–425.

GERARD, R. W., 1938. Anoxia and neural metabolism. *Arch. Neurol. and Psych.*, **40**: 985–996.

HALL, V. E. Personal Communication.

HORSBURGH, D. B., E. L. TATUM AND V. E. HALL, 1940. Chemical properties and physiological actions of Triturus embryonic toxin. *Jour. Pharm. Exp. Ther.*, **68**: 284–291.

MACLEOD, J. J. R., 1934. The control of carbohydrate metabolism. *Bull. Johns Hopkins Hosp.*, **54**: 79–139.

MELLANBY, E., 1935. Snake venoms and cancerous growths. *Ann. Rep. Brit. Empire Cancer Campaign*, **12**: 103–104.

PREGL, F., 1935. Die quantitative organische Mikroanalyse. J. Springer, Berlin.

SPERRY, W. M., AND F. C. BRAND, 1941. Absorption of water by liver slices from "physiological" saline solutions. *Proc. Soc. Exp. Biol. and Med.*, **42**: 147–150.

STILES, H. R., W. H. PETERSON AND E. B. FRED, 1926. A rapid method for the determination of sugar in bacterial cultures. *Jour. Bact.*, **12**: 427–429.

TATUM, E. L., AND G. W. BEADLE, 1938. Development of eye colors in Drosophila: some properties of the hormones concerned. *Jour. Gen. Physiol.*, **22**: 239–253.

TWITTY, V. C., 1937. Experiments on the phenomenon of paralysis produced by a toxin occurring in Triturus embryos. *Jour. Exp. Zool.*, **76**: 67–104.

VAN SLYKE, D. D., 1912. The quantitative determination of aliphatic amino groups. II. *Jour. Biol. Chem.*, **12**: 275–283.